



Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis.

Yann Wallez, Philippe Huber

► To cite this version:

Yann Wallez, Philippe Huber. Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis.. *Biochimica et Biophysica Acta - Molecular Cell Research*, 2008, 1778 (3), pp.794-809. 10.1016/j.bbamem.2007.09.003 . inserm-00433167

HAL Id: inserm-00433167

<https://www.hal.inserm.fr/inserm-00433167>

Submitted on 18 Nov 2009

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ENDOTHELIAL ADHERENS AND TIGHT JUNCTIONS IN VASCULAR HOMEOSTASIS, INFLAMMATION AND ANGIOGENESIS

Yann Wallez^{a,b} and Philippe Huber^{a,*}

^aVascular Pathophysiology laboratory, Inserm U882 38054 Grenoble, France; CEA, 38054

Grenoble, France; Joseph Fourier University 38054 Grenoble, France.

^bPresent address: Burnham Institute for Medical Research and Pathology Department,

University of California San Diego, La Jolla, California, USA.

*Corresponding author: CEA-Grenoble, iRTSV-APV-U882, 17 rue des Martyrs 38054

Grenoble France. Tel: 33-438 78 41 18, fax: 33-438 -78 49 64, e-mail: phuber@cea.fr

Keywords: adherens junction, tight junction, VE-cadherin, claudin, endothelium

ABSTRACT

Endothelial cells lining the vessel wall are connected by adherens, tight and gap junctions. These junctional complexes are related to those found at epithelial junctions but with notable changes in terms of specific molecules and organization. Endothelial junctional proteins play important roles in tissue integrity but also in vascular permeability, leukocyte extravasation and angiogenesis. In this review, we will focus on specific mechanisms of endothelial tight and adherens junctions.

INTRODUCTION

The endothelium is located at the inner side of all vessel types and is constituted by a monolayer of endothelial cells. Interendothelial junctions contain complex junctional structures, namely adherens junctions (AJ), tight junctions (TJ) and gap junctions (GJ), playing pivotal roles in tissue integrity, barrier function and cell-cell communication, respectively. The endothelium constitutes the vascular barrier with regulated permeability properties between the blood and the underlying tissues. Quiescent endothelium may be subjected to stimuli inducing leukocyte extravasation at inflammatory sites and sprouting angiogenesis. Both processes have a strong impact on endothelial cell-cell junctions. In this review, we will focus on endothelial AJ and TJ as well as interendothelial-specific molecules or mechanisms in resting and activated vessels.

Histology of endothelial junctions

The junctional structures located at the endothelial intercellular cleft are related to those found in epithelia; however, their organization is more variable and in most vascular beds their topology is less restricted than in epithelial cells. AJ, TJ and GJ are often intermingled and form a complex zonular system with variations in depth and thickness of the submembrane plaque associated with the junctional structure [1, 2]. As opposed to epithelial cells, GJs are often observed close to the luminal surface. Therefore, the term “apical junction” used to collectively designate epithelial TJ and AJ may not be applied to the endothelium.

Another distinction comes from the difference in cell thickness. With some exceptions, cell body thickness of microvascular endothelium is less than 0.3 μm [2]. Overlapping strands of adjacent endothelial cells form contact domains of 0.5-0.9 μm . However, endothelial cell-cell contacts of some other vessels, including arteries and high endothelial venules, may reach 3-10 μm (Fig. 1) (deduced from [1, 2]). Outside of the electron-dense junctional structures, the intercellular cleft is lined by parallel plasma membranes of neighbor cells separated by 10-20 nm.

Finally, endothelial intercellular domains differ from those of epithelial cells by the absence of desmosomes [2]. The intermediate filaments, constituted in the endothelium by vimentin molecules, are poorly linked to cell-cell contacts. However, as opposed to the situation in epithelia, the vimentin filaments may be linked to endothelial AJ in junctional structures similar to desmosomes, called complexus adherens [3-8]. These structures originally described in lymphatic endothelium may have a broader distribution in the vascular tree.

It must be stressed that interendothelial junctions are dynamic structures, subjected to multiple regulations. Furthermore, leukocytes extravasate at inflammatory sites (mostly in postcapillary venules) either through transcellular or paracellular routes. Extravasation through the intercellular junction is a rapid and regulated process, during which the leukocyte is squeezed in the cleft (diapedesis), followed by rapid junction reformation.

Adhesive proteins located at endothelial cell-cell contacts

A number of proteins exhibiting homophilic adhesive activities are located at interendothelial contacts (Fig. 2). Some of them are specific to endothelial cells (e.g., VE-cadherin [9, 10], claudin-5 [11]) while others are common with epithelial cells (e.g., occludin [12], junctional adhesion molecule (JAM)-A [13], nectins [14, 15], claudins (see references below) and connexins [16]), blood cells (e.g., PECAM/CD31 [17], endothelial cell-selective adhesion molecule (ESAM) [18, 19], JAM-A, -C, CD99 (reviewed in [20]), smooth muscle cells (S-endo-1/CD146 [21]) or mesangial/trophoblast cells (protocadherin (Pcdh)12/VE-cadherin-2 [22]). These proteins may be part of organized junctional structures, such as VE-

cadherin in AJ, claudins and occludin in TJ, or connexins in GJ, while others are independent, such as PECAM, CD99, S-endo-1 or Pcdh12. The JAMs are associated with TJ through intracellular components without being directly involved in TJ strand formation.

Interendothelial adhesive proteins are implicated at different levels in endothelial cell-cell interaction and tissue integrity. Some of them have a dual function as they also participate in leukocyte extravasation via homophilic (PECAM, CD99, JAM-A, -C) or heterophilic (JAM-A, -B, -C) interactions (reviewed in [20]).

THE ENDOTHELIAL ADHERENS JUNCTION

The VE-cadherin-based complex and its physiological role

VE-cadherin is the transmembrane component of endothelial AJ [23]. It is a type II cadherin harboring high adhesive activity [24]. Several biochemical evidences showed that VE-cadherin extracellular domains form hexamers in solution [25, 26]. Electron microscopy data support this view and allowed to propose a model in which VE-cadherin dimers interact in trans through their extracellular domain 1 and VE-cadherin trimers interact in cis via their extracellular domain 4 (Fig. 3) [27].

VE-cadherin intracellular domain is similar to other classical cadherins. It contains a proximal binding site for p120 and p0071, and a distal binding site for β -catenin and plakoglobin (Fig. 4, left). Both β -catenin and plakoglobin are linked to α -catenin, which may further interact with α -actinin and vinculin (see [28] and references therein). The identity of the molecular link for the actin filament anchorage to the cadherin-catenin complex is still a question of debate [29]. As previously indicated, the VE-cadherin complex may associate with the vimentin cytoskeleton in some vascular locations [3-8]. This association is mediated by either plakoglobin or p0071, both interacting with desmoplakin, which in turn associates with vimentin (Fig. 4, right). The VE-cadherin complex also transiently or permanently associates with signaling partners (see below), and with a specific apical-basal polarity complex, through direct interaction with PAR-3 and PAR-6 [30].

The precise mechanisms of VE-cadherin-based junction formation remain mostly unexplored. However, Delanoë-Ayari et al. [31] proposed a model in which VE-cadherin lateral clustering may be achieved by membrane tension and free VE-cadherin molecule diffusion rather than a cytoskeleton-driven mechanism. Consistent with this hypothesis, VE-cadherin clusters forming artificial AJ were observed by assembly of VE-cadherin extracellular domains at the surface of liposomes [32]. As described below, the first cell-cell contacts may be achieved by nectin interaction, which may facilitate cadherin binding.

VE-cadherin was shown to be specifically located at junctions of all endothelium subtypes [9]. VE-cadherin mRNA were observed at the onset of vasculogenesis in the mouse embryo [10]. Its extraendothelial expression has been observed in case of vascular mimicry (the extravillous cytotrophoblasts replacing the spiral artery endothelium [33] or melanoma cells lining tumor vessels [34]), in circulating endothelial progenitors [35] and in podocytes [36].

The VE-cadherin gene yields a single transcript and its promoter contains functional sites for transcription factors such as Ets and Sp1 family members, as well as Tal [37-40]. Although constitutively expressed by endothelial cells, VE-cadherin expression is enhanced by basic FGF in angiogenic situations [41]. Furthermore, Tal, a bHLH transcription factor dramatically upregulated during angiogenesis, activates VE-cadherin transcription [40].

VE-cadherin adhesive properties are essential for optimal homotypic cell interaction; however, the observation that VE-cadherin-deficient embryos retained electron-dense endothelial junctions indicates that some redundancy exist between the several homophilic adhesive proteins located at the endothelial surface [42]. Furthermore, VE-cadherin-deficient

embryos exhibited defective capacities in sprouting angiogenesis resulting in embryonic death at midgestation, suggesting that VE-cadherin has signaling properties required for vascular morphogenesis. The dramatic phenotype of VE-cadherin-deficient embryos was similar to that obtained with partial truncation of VE-cadherin cytoplasmic domain [43]. This feature further points to the implication of VE-cadherin in intracellular signaling.

The contribution of VE-cadherin in neovessel formation has been further demonstrated in adults by experiments using antibodies directed against specific VE-cadherin epitopes, unmasked during angiogenesis, that abrogated angiogenesis and tumor growth in mice [44-46].

Other cadherins, namely N-cadherin [47], T-cadherin [48] and Pcdh12 [22, 49], have also been located in endothelial cells. Albeit abundant in endothelium, N-cadherin is not or poorly located at interendothelial junction [47]. Several converging studies indicate that this molecule promotes heterotypic interactions with perivascular cells such as pericytes [50, 51]. T-cadherin has been identified in endothelium of human aorta [48]. In vitro studies indicate that T-cadherin may participate to various cellular processes, including migration, proliferation and survival [52-55]. Pcdh12 is located at interendothelial junctions, mostly in angiogenic endothelium, and its function is presently unknown [22, 49].

β -catenin trafficking in endothelial cells

As described for epithelial cells, β -catenin may translocate to the nucleus and activate several genes including cyclin D1 and myc whose products induce entry in the cell cycle [56].

During human embryonic development, β -catenin was detected in endothelial cell nuclei and cytoplasm of capillaries (in particular in the brain), arteries and veins [57-59]. In quiescent endothelial cells of adults, β -catenin is detectable neither in the nucleus nor in the cytoplasm, but appears to be concentrated at cell-cell contacts. Conversely, β -catenin is often observed in the nucleus and cytoplasm of endothelial cells during pathological angiogenesis or vascular remodeling. Consistently, intracellular (nuclear or cytosolic, as opposed to junctional) accumulation of β -catenin correlates with BrDU incorporation, which therefore links the presence of intracellular β -catenin to a proliferative state.

The conditional inactivation of β -catenin in the endothelium leads to morphological alterations of vessels, increased vascular permeability and eventually results in embryonic lethality at mid-gestation [60, 61]. In endothelial cells devoid of β -catenin, plakoglobin solely binds to VE-cadherin. As opposed to β -catenin, plakoglobin may bind to either α -catenin or desmoplakin and vimentin. Therefore, the VE-cadherin complex may interact more strongly with the vimentin cytoskeleton in this context.

Altogether, these features suggest that physiological regulation of endothelial junctions depends on (i) a subtle balance between β -catenin and plakoglobin binding to VE-cadherin and (ii) the proportion between intracellular and junctional β -catenin.

Regulation of VE-cadherin barrier function

Beside its role in maintaining endothelium integrity, VE-cadherin participates in the control of vascular permeability and leukocyte transmigration, as documented by injection of VE-cadherin function blocking antibodies in the mouse [62-64]. During leukocyte transmigration, VE-cadherin-catenin complexes are pushed aside to facilitate diapedesis [65-67]. An alternative mechanism has been described that implicates the degradation of VE-cadherin molecules by various proteases, as discussed below.

VEGF (vascular endothelial growth factor) is a pleiotropic cytokine relatively specific to endothelial cells or their progenitors inducing vascular permeability, cell survival or proliferation in a context-dependent manner [68]. In confluent endothelial cells, VEGF induces a rapid and transient tyrosine phosphorylation of VE-cadherin as well as β -catenin

and plakoglobin [69]. These molecular events are accompanied by increased paracellular permeability and decreased association of the VE-cadherin complex with the cytoskeleton. Furthermore, VEGFR2, the receptor transducing VEGF signals in endothelial cells, interacts with the VE-cadherin complex, probably through β -catenin [43, 70]. VE-cadherin tyrosine phosphorylation and association to VEGFR2 was confirmed in vivo in a model of physiological angiogenesis dependent on VEGF [71]. Src tyrosine kinase is constantly associated with VE-cadherin [71]. Chou et al. [72] showed that Src also associates with VEGFR2 and gets activated upon VEGF stimulation. Src inhibitors prevent VEGF-induced VE-cadherin phosphorylation, thereby indicating that Src is an obligatory mediator in this pathway [71]. Furthermore, Src directly phosphorylates VE-cadherin cytoplasmic domain on tyrosine 685 (Fig. 5A) [73]. The functional consequences of Y685 phosphorylation remain elusive, although a direct binding of C-terminal Src kinase (Csk) to phospho-Y685 has been reported (see below) [74]. Other VE-cadherin tyrosine phosphorylation sites (Y658 and Y731) have been described in correlation with increased permeability [75].

Recently, Gavard et al. [76] revealed an additional role of VEGF in VE-cadherin endocytosis and thus junction opening (Fig. 5B). VEGF induces the activation of the exchange factor Vav2 via Src. Vav2 activates Rac, which in turn activates PAK kinase, enabling phosphorylation of VE-cadherin cytoplasmic domain on serine 665. Phospho-S665 VE-cadherin becomes a docking site for β -arrestin-2, the binding of which triggers VE-cadherin endocytosis.

VEGF is not the sole endothelial effector inducing tyrosine phosphorylation of the VE-cadherin complex. Other permeability factors, including histamine, tumor necrosis factor (TNF)- α , platelet-activating factor (PAF) as well as activated neutrophils or integrin engagement phosphorylate VE-cadherin [77-83].

In quiescent vessels, VE-cadherin is in a dephosphorylated state. Several protein tyrosine phosphatases are associated with VE-cadherin: Dep-1 [84], VE-PTP [85], PTP- μ [86, 87] and SHP-2 [88]. VE-PTP and PTP- μ , two integral membrane proteins, directly interact with VE-cadherin extracellular and intracellular domains, respectively. Both phosphatases were shown to dephosphorylate VE-cadherin after VEGF stimulation, leading to increased barrier function.

VE-cadherin extracellular domain is highly sensitive to proteolysis and a 90 kDa-related protein corresponding to its extracellular domain is often detected in tissue extracts [71].

Whereas VE-cadherin cleavage by activated neutrophils has been a question of debate, some evidences indicate that proteases liberated by or at the surface of stimulated neutrophils [89], namely neutrophil elastase and cathepsin G [90] and probably others [91], cleave VE-cadherin, thereby facilitating neutrophil transmigration.

VE-cadherin degradation by matrix metalloproteinase, such as MMP-2, -7 and -9, has been documented in several settings, including apoptosis, diabetes and Dengue virus infection [92-96].

VE-cadherin is also degraded by clathrin-dependent endocytosis and lysosomal targeting by a mechanism involving p120 [97-100]. P120 downregulation in endothelial cells induces a dramatic decrease in barrier function associated with a loss of VE-cadherin protein levels. P120 may thus be an intracellular regulator of VE-cadherin degradation.

In conclusion, several pathways co-exist to reduce VE-cadherin-dependent barrier function: VE-cadherin/catenins complex dissociation from the cytoskeleton, VE-cadherin internalization, junctional movements and VE-cadherin degradation.

Contact inhibition of cell proliferation as a consequence of junction formation

The essential role of VE-cadherin in contact inhibition was revealed by two in vitro experiments. In a first study, Dejana's group showed that proliferation was inhibited when

endothelial cells were seeded on dishes onto which the extracellular VE-cadherin domain had been adsorbed [101]. More recently, the same group reported that VE-cadherin-deficient endothelial cells had lost their contact inhibition and reached increased cell density [84].

Several signaling pathways were identified that may explain the growth inhibition controlled by VE-cadherin.

(1) VE-cadherin might sequester β -catenin at the membrane, thereby preventing transcriptional activation of proliferative genes. Interestingly, a study exploited this property by ectopically expressing the VE-cadherin cytoplasmic domain in tumor cells, to successfully block β -catenin transcriptional activity and specifically kill these cells [102].

(2) VE-cadherin binding to VEGFR2 leads to a dramatic decrease in VEGFR2 tyrosine phosphorylation [84]. Consequently, both MAP kinase activation and cellular proliferation are reduced. The protein tyrosine phosphatase Dep1, associated to the VE-cadherin complex, might be responsible for VEGFR2 dephosphorylation (Fig. 6). More recently, the mechanism by which VE-cadherin attenuates VEGF proliferative signal has been further deciphered [103]. Junctional VE-cadherin reduces VEGFR2 internalization after VEGF activation and decreases receptor signaling from endosomal compartments by a Dep1-dependent mechanism. Conversely, in sparse cells, VEGFR2 is internalized faster and its proliferative activity lasts longer.

(3) VE-cadherin interacts with Shc, a protein known to activate the MAP kinase cascade, after stimulation with VEGF [104]. This binding leads to Shc dephosphorylation probably by junctional VE-cadherin-associated phosphatases. Phospho-Shc is able to couple Grb2-Sos to Ras, which eventually activates the MAP kinases (Fig. 6) [105]. Therefore, junctional docking of Shc and its subsequent dephosphorylation may inhibit VEGF proliferative activity.

(4) The protein tyrosine kinase Csk (C-terminal Src kinase) is a negative regulator of the Src family kinases that inactivates these enzymes by phosphorylation of their inhibitory tyrosine, thereby imposing a locked conformation [106]. Analysis of chimeric embryos containing Csk-deficient and wild type cells revealed defects in angiogenic sprouting and vascular remodeling, a phenotype reminiscent of VE-cadherin-deficient embryos [107]. Csk binds to VE-cadherin cytoplasmic domain when Y685 is phosphorylated (Fig. 5A) [74] and Csk siRNA inhibition in endothelial cells increases proliferation. It is thus likely that VE-cadherin participates in cell contact inhibition by recruitment of Csk and inhibition of Src activity.

(5) Finally, by culturing endothelial cells on patterned substrates, Nelson and Chen [108] showed that VE-cadherin may inhibit proliferation by actively decreasing cell spreading. By preventing the VE-cadherin-induced change in morphology, the authors revealed that VE-cadherin also elicit a growth signal mediated by actin cytoskeleton tension.

VE-cadherin implication in VEGF survival signaling and the endothelial hemodynamic force response

Quiescent endothelial cells are resistant to harmful stimuli, such as serum deprivation. Similar to other cadherin members, VE-cadherin protects endothelial cells from serum deprivation-induced apoptosis. Yet, VEGF-induced phosphorylation of PI3 kinase and subsequent activation of the serine-threonine kinase Akt is enhanced by VE-cadherin and its binding to the VEGFR2 complex (Fig. 6) [43].

In addition, VE-cadherin induces Gas1 (growth arrest-specific 1) expression [109]. This protein efficiently protects cells from apoptosis. Interestingly, VE-cadherin-blocking antibodies prevent VEGF-induced stimulation of Gas1 expression, suggesting that junctional assembly of VE-cadherin is required for Gas1 induction.

Therefore, VE-cadherin acts both as a junctional sensor and a molecular switch, directing VEGF signaling towards proliferation or survival.

Endothelial cells are also exposed to and activated by the bloodstream. Hemodynamic forces are variable along the vascular tree and induce a complex response in the endothelium, including the induction or repression of several genes as well as modifications in cell morphology. Junction resistance to flow stress is dependent upon VE-cadherin, and more specifically upon its binding to plakoglobin [110]. Furthermore, loss of VE-cadherin precludes transcriptional activation of a shear stress-responsive promoter. In fact, VEGFR2, VE-cadherin together with PECAM/CD31 form a mechanosensor complex that is sufficient to activate a flow-response to heterologous cells [111, 112].

VE-cadherin and cytoskeleton organization through G protein activation

Cadherins are good candidates as mediator of cytoskeletal organization modifications, such as those observed during epithelio-mesenchymal transition. An increasing number of studies indicates that cadherin engagement induces activation of Rho family GTPases.

Rac activity is associated with endothelial junction strengthening. Yet, its inhibition induces endothelial junction disruption [113]. Rac may act by promoting actin polymerization or by coupling the VE-cadherin complex to the cytoskeleton [114].

A recent study shows that acute hypoxia transiently inhibits Rac1, leading to RhoA activation, actin stress fiber formation, adherens junction opening and eventually increased endothelial permeability. Conversely, re-oxygenation strongly activates Rac1 and restores the cortical location of actin filament by inhibiting RhoA [115].

The endothelium is separated from interstitial collagens by a basal lamina constituted by several proteins, including laminins. Laminin I induces a sustained activation of Rac, correlated with endothelial quiescence, while collagen I, to which sprouting cells are exposed, triggers adherens junction disruption and capillary morphogenesis associated with and dependent of Rho activation [116].

Intriguingly, a recent study reports the activation of Rac1 in VEGF-treated endothelial cells, which transiently prevents VEGF-induced paracellular permeability [117]. A dominant-negative form of Rac1 abolishes the transient barrier effect, whereas the established and sustained effect of VEGF is unmodified.

Numerous reports indicate that Rac activity increases the endothelial barrier. However, this notion was challenged by van Wetering et al. [118], who showed that expression of constitutively active Rac in endothelial cells caused a rapid and ROS (reactive oxygen species)-dependent disruption of cell junctions. The same group further demonstrated that activation of Rac and subsequent production of ROS resulted in β -catenin phosphorylation by the tyrosine kinase Pyk2 [119]. As mentioned above, Rac also participates in VE-cadherin internalization after VEGF stimulation [76].

Altogether, these results show that Rac is involved in several signaling pathways leading to either endothelial barrier reinforcement or increased permeability.

Whether it is clearly demonstrated that Rho and Rac are potent effectors of VE-cadherin adhesive activity, the reverse is also true. Hence, VE-cadherin re-expression in VE-cadherin-deficient endothelial cells increases Rac activity by augmenting the expression of Rac-specific guanosine-exchange factor Tiam1 [120]. Furthermore, VE-cadherin re-expression increases the membrane-associated pools of Tiam1, Rac and its effector PAK (p-21 activated kinase). These properties are lost when VE-cadherin lacks domains interacting with p120 or β -catenin.

VE-cadherin also regulates RhoA, which in turn transmits signals via the actin cytoskeleton to adhesive proteins implicated in cell-matrix attachment [108, 121]. As previously mentioned, following cell-cell contacts, this signaling may limit endothelial cell spreading.

Hence, VE-cadherin, by interfering with small G-protein activity, may act on cytoskeleton organization, cell spreading and matrix adhesion.

The GTPase Cdc42 also controls VE-cadherin activity. Indeed, a dominant-negative mutant of Cdc42 dramatically decreases endothelial junction reformation, following an inflammatory stimulation like thrombin [122]. The same group further demonstrated that Cdc42 acts on adherens junctions by controlling the binding of α -catenin to the β -catenin/VE-cadherin complex [123]. Remarkably, the cytoplasmic domain of extrajunctional VE-cadherin is capable to induce formation of extended membrane protrusions by a Cdc42-dependent mechanism [124]. Hence, Cdc42 controls VE-cadherin protrusive activity by an inside-out signaling pathway utilizing actin polymerization. This mechanism, together with the potent heterophilic adhesion of VE-cadherin with fibrin [125], may play a pivotal role in capillary morphogenesis.

Cyclic AMP (cAMP) is a second messenger acting downstream of G-protein-coupled receptors, whose production increases endothelial barrier function [126]. Receptor agonists, such as prostacyclins or prostaglandins, attenuate the hyperpermeability induced by inflammatory stimuli [127]. A recent study shed light on the role of Rap1, a Ras family GTPase, in this process [128]. Elevated levels of intracellular cAMP enable the activation of the exchange factor Epac, which is a specific activator of Rap1. Epac or Rap1 activation decreases permeability by promoting the extension of VE-cadherin-based junctions (Fig. 7) [129]. Another report indicates that the homophilic engagement of VE-cadherin is sufficient to activate Rap1, by a mechanism dependent on the adaptor protein MAGI-I [130]. This protein, probably recruited at the junction by β -catenin, is associated with the exchange factor PDZ-GEF1, which is a direct Rap1 activator (Fig. 7). Hence, first homophilic VE-cadherin contacts may autonomously enhance adherens junction assembly. Both the cAMP-Epac-Rap1 and the MAGI-I-PDZ-GEF1-Rap1 pathways may interfere with VE-cadherin-based junctions by controlling the cortical actin cytoskeleton.

Although the regulation mechanisms linking AJ and the cytoskeleton are probably not fully elucidated, it is possible to conclude that the VE-cadherin complex is an actor of vascular morphogenesis through actin remodeling, as well as an integrator of endothelial stimuli and physiological state.

THE ENDOTHELIAL TIGHT JUNCTION

TJs show considerable variability among different segments of the vascular tree [2]. This disparity constitutes a major evidence of vascular bed differentiation of endothelial cells and has a strong impact on vascular permeability and leukocyte extravasation. Variations concern the complexity degree of the occluding strands as well as TJ composition.

Large artery endothelial cells, which are exposed to high flow rates, display a well-developed system of TJ. Within the microvasculature, TJ are less complex in capillaries than in arterioles, and even less in venules. As previously mentioned, post-capillary venules are the primary site of leukocyte extravasation, and accordingly, they display a high content of permeability mediator receptors, such as those for histamine, serotonin and bradykinin. At the opposite, the blood brain barrier (BBB) and the blood retinal barrier (BRB) are particularly rich in TJ and endothelial TJs have been mostly studied in these locations (as reviews, see [2, 28, 131-135]). It is currently admitted that the BBB/BRB TJ phenotype is induced by 2 perivascular cell types: the astrocytes through their cellular processes (end foot) and the closely associated pericytes. Both cells produce factors, including angiopoietin-1, TGF β and probably other uncharacterized molecules, that are required to induce the barrier phenotype and [136-138].

As discussed below, some of TJ components are specific to endothelial cells. In addition, the same molecules might be differentially assembled and regulated in endothelial versus epithelial TJs. As previously mentioned, we will concentrate herein on TJ molecules and mechanisms specific to endothelial cells. The general organization of TJs is described in chapters 2-3 and in previous reviews [28, 139]. It should be noted that information regarding TJ component properties mostly derives from studies on epithelial cells because of lack of convenient endothelial cell models retaining TJ.

Claudins, occludin and the submembrane TJ complex

Claudin family members are the major TJ transmembrane constituents, exhibiting homophilic and heterophilic (with other claudin subtypes) adhesive activities through their extracellular domains and forming the TJ strands [135]. As previously mentioned, endothelial TJs specifically and highly express claudin-5, with a few exceptions [140]. Claudin-5-deficient mice have selective impairment in BBB function for molecules smaller than 800 Da [141]. This phenotype suggests a partial redundancy between claudin subtypes. Yet, claudin-3 and -11 have also been characterized in endothelial cells [142-145]. Initial identification of claudin-1 and -12 [141, 146] in the endothelium is now largely questioned and was not confirmed. Beside their barrier function, claudins also behave as specific channels and their diversity reflects their differential function in paracellular transport [147]. A number of pathological settings associated with decreased BBB function were correlated with lower claudin expression, thereby indicating that claudins may be therapeutic targets in brain edema lesions [144, 146, 148-152].

Another transmembrane component of TJ strands is occludin. Although not necessary for TJ strand formation, occludin is associated with increased TJ barrier function. In the endothelium, this molecule is specifically expressed in the BBB and the BRB [12] and its expression is increased with pericyte-derived angiopoietin-1 [136]. Whereas occludin downregulation has been observed in various disease state associated with BBB or BRB disruption, including stroke, diabetes as well as hypoxia/aglycemia [153-158]. Nevertheless, the fact that genetic ablation of occludin does not affect endothelial TJ organization or permeability is intriguing and may indicate differential functions in man and mouse [159].

Decreased occludin contents together with increased paracellular permeability were observed in VEGF-treated retinal endothelial cells by a proteolytic mechanism depending on urokinase plasminogen activator (Fig. 8) [153, 160]. Occludin proteolysis by metalloproteinases was also observed after protein tyrosine phosphatase inhibition or monocyte diapedesis [161, 162]. These features suggest that occludin degradation may be one of the mechanisms increasing vascular permeability. VEGF activation also leads to occludin phosphorylation on serine/threonine residues through PKC activation in correlation with increased permeability (Fig. 8) [163, 164]. Upon VEGF treatment, occludin is phosphorylated on multiple serine and threonine residues, however the identification of the precise phosphorylation sites and their molecular function remain elusive [154]. Other factors, namely lysophosphatidic acid, histamine, oxidized phospholipids, monocyte chemoattractant protein-1 (MCP-1 or CCL-2) or shear-stress, induce both occludin phosphorylation on serine/threonine residues and increased permeability (Fig. 8) [165-168]. But paradoxically, angiotensin-2 was shown to increase blood brain barrier function in correlation with increased threonine phosphorylation of occludin together with its mobilization to lipid rafts (Fig. 8) [169]. Thus, the functional consequences of occludin phosphorylation may be phospho-site-dependent. Stamatovic et al. [168] demonstrated that MCP-1, in addition to occludin, also targets ZO-1, ZO-2 (see below) and claudin-5 phosphorylation on serine/threonine residues by a signaling pathway involving Rho and PKC α . Occludin may also be phosphorylated on tyrosine residues following cerebral ischemia and angiotensin-2 has a negative impact on

occludin tyrosine phosphorylation level (Fig. 8) [168, 170]. In conclusion, inflammatory or angiogenic mediators alter BBB function in part by acting on occludin integrity, localization or phosphorylation level. Conversely, hydrocortisone treatment, through its anti-inflammatory properties, increased occludin levels and barrier properties of retinal endothelial cells [171].

Claudins and occludin are linked to numerous intracellular partners, including ZO-1, -2 and -3, AF-6/afadin, PAR-3, cingulin and 7H6 antigen, also present in epithelial TJs [28]. These proteins form a molecular complex at the submembrane side of TJs. Detailed properties of these proteins and their molecular interactions are reviewed in other chapters and reviews [149]. Interestingly, two versions of ZO-1 resulting from alternative splicing may be found. An 80 amino-acid domain, called α , may be inserted in the central part of the protein. Whereas the ZO-1 α^+ variant is found in most epithelial cells, the ZO-1 α^- isoform is only observed in endothelial cells, Sertoli cells and podocytes of kidney glomeruli [172-174]. In general, the ZO-1 α^- isoform characterizes more dynamic junctions. Symplekin, another protein located in the TJ complex of some epithelia, is absent in endothelial cells [175].

The JAMs

JAM-A, -B, and -C constitute a family of transmembrane adhesive proteins belonging to Ig superfamily that colocalize with TJ, although not included in TJ strands [20]. Of note, JAM-B localization at TJ has been challenged [176]. JAM-A is located in epithelial and endothelial intercellular junctions and at the surface of platelets and leukocytes [13, 177], while JAM-C expression is restricted to endothelial cells of lymphatic sinuses and high endothelial venules of lymphatic organs, as well as smooth muscle cells, fibroblasts and some blood cells; however its tissue distribution varies between mouse and human (see [178] and references therein and [179]). JAM-B is expressed at interendothelial junctions [180] but its tissue distribution is less documented than the two other members.

JAM-A develops homophilic adhesive activity, suggesting that it may mediate endothelial cell-cell interaction. Its barrier function is further demonstrated by use of blocking antibodies or peptides [181, 182]. Furthermore, the presence of JAM-A at intercellular junctions reduces paracellular permeability [13]. Intracellularly, it interacts with several members of TJ complex (see [183], for more details), as well as the PAR-3/aPKC/PAR-6 polarity complex, suggesting that JAM-A may be involved in the establishment of apical-basal polarity. Most recent studies on JAM-A are focused on its role in leukocyte transmigration. In inflammatory conditions, JAM-A is redistributed at the apical surface [184, 185]. JAM-A, together with PECAM, CD99 [20] and other JAMs (see below), facilitates leukocyte transmigration through the intercellular cleft. During diapedesis, JAM-A binds heterophilically to $\alpha_L\beta_2$ integrin located on leukocytes [186]. The phenotype of JAM-A-deficient mice confirmed its activity in neutrophil transmigration and unveiled other functions including the restriction of dendritic cell trafficking in lymph nodes or its participation in bFGF-induced angiogenesis [187-190].

JAM-C develops homophilic binding at interendothelial junctions, where it may also interact, even with higher affinity, with JAM-B [191, 192]. As opposed to JAM-A, junctional localization of JAM-C increases paracellular permeability, possibly by modulating VE-cadherin cell-cell contacts [176, 193]. JAM-C promotes neutrophil transmigration via its binding to the leukocyte integrin $\alpha_M\beta_2$ [192], but this activity is lost under shear-stress conditions, suggesting a weak interaction [194]. Two reports indicate that tumor cells also express JAM-C at their surface [195, 196]; this feature may promote the metastatic potential of these cells by facilitating their adhesion to endothelial cells via homophilic binding and their subsequent transmigration. JAM-C also regulates tumor cell migration by regulating integrin activity [197]. JAM-C-deficient mice exhibit growth retardation and pneumonia causing poor survival of the mice [198]. Additionally, the number of circulating granulocytes

is increased, which seems to be caused by loss of endothelial JAM-C as its rescue in endothelial cells is sufficient to restore homeostasis and better survival.

ESAM is a transmembrane Ig protein related to JAMs [18]. Its expression is restricted to endothelial cell TJs and to the surface of activated platelets. ESAM mediates homophilic binding and its only known cytoplasmic binding partner is MAGI-I [199]. ESAM-deficient mice showed defective tumor angiogenesis, whereas physiological angiogenesis was normal [200]. Endothelial ESAM also participates in neutrophil extravasation by a mechanism involving Rho activation and TJ destabilization [201].

Collectively, these data indicate that JAM/ESAM family members have pivotal and selective functions in leukocyte trafficking and may also influence TJ dynamics.

Nectins

Nectins form another group of cell-cell adhesion molecules belonging to the Ig superfamily [15, 202, 203]. Four members have been described, nectin 1-4, as well as five nectin-like molecules, Necl 1-5. Nectin C-terminus interacts with the PDZ domain of afadin/AF-6, an actin filament-binding protein, which connects them to the actin cytoskeleton (Fig. 4). Nectin/afadin were found associated with TJ and AJ proteins. They participate in the initial step of junction formation and play a fundamental role in the establishment of polarity. The nectin-like molecules do not exhibit a PDZ-binding motif and do not interact with afadin.

So far, only nectin-2 and Necl-5 were identified in endothelial cells [14, 204]. Nectin-2 is an α -herpes receptor and Necl-5 is the poliovirus receptor [202] and thus both receptors may be involved in viral dissemination. Nectin-2 develop homophilic as well as heterophilic interactions with nectin-3, while Necl-5 does not develop homophilic binding but interacts with nectin-3 [15]. Necl-5 is a potential receptor for DNAM-1/CD226, a transmembrane glycoprotein expressed on a subset of B cells and all T cells, natural killer cells, monocytes and platelets. Necl-5 facilitates monocyte transmigration through endothelial monolayers and may thus participate in inflammatory processes in vivo. Nectin-2, which is also a DNAM-1 ligand in other cell types, does not seem to play a similar role in endothelial cells [14].

FUTURE DIRECTIONS

In the recent years, the number of adhesive proteins at interendothelial junctions has stabilized and their contribution to barrier function has been relatively well established. Nevertheless, a comprehensive view of the proteins located on the cytoplasmic side of AJ and TJ is still missing. For example, after years of investigation, the molecular link between the actin cytoskeleton and the AJ remains elusive. The molecular network in both junction types is probably more complex and variable according to location and pathophysiological state than hitherto thought. A number of endothelial adhesive proteins are implicated in leukocyte adhesion and transmigration; their specific and sequential actions in these processes remain to be clarified. Finally, some of these proteins have demonstrated function in angiogenesis or cell migration through mechanisms that need to be fully investigated.

ACKNOWLEDGEMENTS

YW was supported by grants from the Rhône-Alpes regional council.

REFERENCES

- [1] J.A.G. Rhodin, Histology, Oxford University Press, New York, 1974.
- [2] W.W. Franke, P. Cowin, C. Grund, C. Kuhn, H.P. Kapprell, The endothelial junction: the plaque and its component., in: N. Simionescu, M. Simionescu (Eds.), Endothelial cell biology in health and diseases, Plenum publishing corporation, New York, 1988, pp. 147-166.
- [3] M. Schmelz, W.W. Franke, Complexus adhaerentes, a new group of desmoplakin-containing junctions in endothelial cells: the syndesmos connecting retothelial cells of lymph nodes, *Eur J Cell Biol* 61 (1993) 274-289.
- [4] M. Schmelz, R. Moll, C. Kuhn, W.W. Franke, Complexus adhaerentes, a new group of desmoplakin-containing junctions in endothelial cells: II. Different types of lymphatic vessels, *Differentiation; research in biological diversity* 57 (1994) 97-117.
- [5] A.P. Kowalczyk, P. Navarro, E. Dejana, E.A. Bornslaeger, K.J. Green, D.S. Kopp, J.E. Borgwardt, VE-cadherin and desmoplakin are assembled into dermal microvascular endothelial intercellular junctions: a pivotal role for plakoglobin in the recruitment of desmoplakin to intercellular junctions, *J Cell Sci* 111 (1998) 3045-3057.
- [6] K. Venkiteswaran, K. Xiao, S. Summers, C.C. Calkins, P.A. Vincent, K. Pumiglia, A.P. Kowalczyk, Regulation of endothelial barrier function and growth by VE-cadherin, plakoglobin, and beta-catenin, *Am J Physiol Cell Physiol* 283 (2002) C811-821.
- [7] C.C. Calkins, B.L. Hoepner, C.M. Law, M.R. Novak, S.V. Setzer, M. Hatzfeld, A.P. Kowalczyk, The Armadillo family protein p0071 is a VE-cadherin- and desmoplakin-binding protein, *J Biol Chem* 278 (2003) 1774-1783.
- [8] B. Hammerling, C. Grund, J. Boda-Heggemann, R. Moll, W.W. Franke, The complexus adhaerens of mammalian lymphatic endothelia revisited: a junction even more complex than hitherto thought, *Cell and tissue research* 324 (2006) 55-67.
- [9] M.G. Lampugnani, M. Resnati, M. Raiteri, R. Pigott, A. Pisacane, G. Houen, L.P. Ruco, E. Dejana, A novel endothelial-specific membrane protein is a marker of cell-cell contacts, *J Cell Biol* 118 (1992) 1511-1522.
- [10] G. Breier, F. Breviario, L. Caveda, R. Berthier, H. Schnurch, U. Gotsch, D. Vestweber, W. Risau, E. Dejana, Molecular cloning and expression of murine vascular endothelial- cadherin in early stage development of cardiovascular system, *Blood* 87 (1996) 630-641.
- [11] K. Morita, H. Sasaki, M. Furuse, S. Tsukita, Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells, *J Cell Biol* 147 (1999) 185-194.
- [12] T. Hirase, J.M. Staddon, M. Saitou, Y. Ando-Akatsuka, M. Itoh, M. Furuse, K. Fujimoto, S. Tsukita, L.L. Rubin, Occludin as a possible determinant of tight junction permeability in endothelial cells, *J Cell Sci* 110 (Pt 14) (1997) 1603-1613.
- [13] I. Martin-Padura, S. Lostaglio, M. Schneemann, L. Williams, M. Romano, P. Fruscella, C. Panzeri, A. Stoppacciaro, L. Ruco, A. Villa, D. Simmons, E. Dejana, Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration, *J Cell Biol* 142 (1998) 117-127.
- [14] N. Reymond, A.M. Imbert, E. Devilard, S. Fabre, C. Chabannon, L. Xerri, C. Farnarier, C. Cantoni, C. Bottino, A. Moretta, P. Dubreuil, M. Lopez, DNAM-1 and PVR regulate monocyte migration through endothelial junctions, *The Journal of experimental medicine* 199 (2004) 1331-1341.
- [15] H. Ogita, Y. Takai, Nectins and nectin-like molecules: roles in cell adhesion, polarization, movement, and proliferation, *IUBMB life* 58 (2006) 334-343.

- [16] C. de Wit, S.E. Wolfle, B. Hopfl, Connexin-dependent communication within the vascular wall: contribution to the control of arteriolar diameter, *Advances in cardiology* 42 (2006) 268-283.
- [17] P.J. Newman, The biology of PECAM-1, *J Clin Invest* 99 (1997) 3-8.
- [18] K. Hirata, T. Ishida, K. Penta, M. Rezaee, E. Yang, J. Wohlgemuth, T. Quertermous, Cloning of an immunoglobulin family adhesion molecule selectively expressed by endothelial cells, *J Biol Chem* 276 (2001) 16223-16231.
- [19] I. Nasdala, K. Wolburg-Buchholz, H. Wolburg, A. Kuhn, K. Ebnet, G. Brachtendorf, U. Samulowitz, B. Kuster, B. Engelhardt, D. Vestweber, S. Butz, A transmembrane tight junction protein selectively expressed on endothelial cells and platelets, *J Biol Chem* 277 (2002) 16294-16303.
- [20] W.A. Muller, Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response, *Trends Immunol* 24 (2003) 327-334.
- [21] N. Bardin, F. Anfosso, J.M. Masse, E. Cramer, F. Sabatier, A. Le Bivic, J. Sampol, F. Dignat-George, Identification of CD146 as a component of the endothelial junction involved in the control of cell-cell cohesion, *Blood* 98 (2001) 3677-3684.
- [22] C. Rampon, M.H. Prandini, S. Bouillot, H. Pointu, E. Tillet, R. Frank, M. Vernet, P. Huber, Protocadherin 12 (VE-cadherin 2) is expressed in endothelial, trophoblast, and mesangial cells, *Exp Cell Res* 302 (2005) 48-60.
- [23] M.G. Lampugnani, M. Corada, L. Caveda, F. Breviario, O. Ayalon, B. Geiger, E. Dejana, The molecular organization of endothelial cell to cell junctions: differential association of plakoglobin, beta-catenin, and alpha- catenin with vascular endothelial cadherin (VE-cadherin), *J Cell Biol* 129 (1995) 203-217.
- [24] P. Panorchan, J.P. George, D. Wirtz, Probing intercellular interactions between vascular endothelial cadherin pairs at single-molecule resolution and in living cells, *J Mol Biol* 358 (2006) 665-674.
- [25] P. Legrand, S. Bibert, M. Jaquinod, C. Ebel, E. Hewat, F. Vincent, C. Vanbelle, E. Concord, T. Vernet, D. Gulino, Self-assembly of the vascular endothelial cadherin ectodomain in a Ca²⁺-dependent hexameric structure, *J Biol Chem* 276 (2001) 3581-3588.
- [26] S. Bibert, M. Jaquinod, E. Concord, C. Ebel, E. Hewat, C. Vanbelle, P. Legrand, M. Weidenhaupt, T. Vernet, D. Gulino-Debrac, Synergy between extracellular modules of vascular endothelial cadherin promotes homotypic hexameric interactions, *J Biol Chem* 277 (2002) 12790-12801.
- [27] E.A. Hewat, C. Durmort, L. Jacquamet, E. Concord, D. Gulino-Debrac, Architecture of the VE-cadherin hexamer, *J Mol Biol* 365 (2007) 744-751.
- [28] G. Bazzoni, E. Dejana, Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis, *Physiol Rev* 84 (2004) 869-901.
- [29] S. Yamada, S. Pokutta, F. Drees, W.I. Weis, W.J. Nelson, Deconstructing the cadherin-catenin-actin complex, *Cell* 123 (2005) 889-901.
- [30] S. Iden, D. Rehder, B. August, A. Suzuki, K. Wolburg-Buchholz, H. Wolburg, S. Ohno, J. Behrens, D. Vestweber, K. Ebnet, A distinct PAR complex associates physically with VE-cadherin in vertebrate endothelial cells, *EMBO reports* 7 (2006) 1239-1246.
- [31] H. Delanoe-Ayari, R. Al Kurdi, M. Vallade, D. Gulino-Debrac, D. Riveline, Membrane and acto-myosin tension promote clustering of adhesion proteins, *Proc Natl Acad Sci U S A* 101 (2004) 2229-2234.
- [32] O. Lambert, J.C. Taveau, J.L. Him, R. Al Kurdi, D. Gulino-Debrac, A. Brisson, The basic framework of VE-cadherin junctions revealed by cryo-EM, *J Mol Biol* 346 (2005) 1193-1196.

- [33] Y. Zhou, S.J. Fisher, M. Janatpour, O. Genbacev, E. Dejana, M. Wheelock, C.H. Damsky, Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion?, *J Clin Invest* 99 (1997) 2139-2151.
- [34] M.J. Hendrix, E.A. Seftor, P.S. Meltzer, L.M. Gardner, A.R. Hess, D.A. Kirschmann, G.C. Schatteman, R.E. Seftor, Expression and functional significance of VE-cadherin in aggressive human melanoma cells: role in vasculogenic mimicry, *Proc Natl Acad Sci U S A* 98 (2001) 8018-8023.
- [35] M. Peichev, A.J. Naiyer, D. Pereira, Z. Zhu, W.J. Lane, M. Williams, M.C. Oz, D.J. Hicklin, L. Witte, M.A. Moore, S. Rafii, Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors, *Blood* 95 (2000) 952-958.
- [36] C.D. Cohen, A. Klingenhoff, A. Boucherot, A. Nitsche, A. Henger, B. Brunner, H. Schmid, M. Merkle, M.A. Saleem, K.P. Koller, T. Werner, H.J. Grone, P.J. Nelson, M. Kretzler, Comparative promoter analysis allows de novo identification of specialized cell junction-associated proteins, *Proc Natl Acad Sci U S A* 103 (2006) 5682-5687.
- [37] P. Huber, J. Dalmon, J. Engiles, F. Breviario, S. Gory, L.D. Siracusa, A.M. Buchberg, E. Dejana, Genomic structure and chromosomal mapping of the mouse VE-cadherin gene (*Cdh5*), *Genomics* 32 (1996) 21-28.
- [38] S. Gory, J. Dalmon, M.H. Prandini, T. Kortulewski, Y. de Launoit, P. Huber, Requirement of a GT box (Sp1 site) and two Ets binding sites for vascular endothelial cadherin gene transcription, *J Biol Chem* 273 (1998) 6750-6755.
- [39] E. Lelievre, V. Mattot, P. Huber, B. Vandenbunder, F. Soncin, ETS1 lowers capillary endothelial cell density at confluence and induces the expression of VE-cadherin, *Oncogene* 19 (2000) 2438-2446.
- [40] V. Deleuze, E. Chalhoub, R. El-Hajj, C. Dohet, M. Le Clech, P.O. Couraud, P. Huber, D. Mathieu, TAL-1/SCL and its partners E47 and LMO2 up-regulate VE-cadherin expression in endothelial cells, *Mol Cell Biol* 27 (2007) 2687-2697.
- [41] M.H. Prandini, I. Dreher, S. Bouillot, S. Benkerri, T. Moll, P. Huber, The human VE-cadherin promoter is subjected to organ-specific regulation and is activated in tumour angiogenesis, *Oncogene* 24 (2005) 2992-3001.
- [42] S. Gory-Fauré, M.H. Prandini, H. Pointu, V. Roullot, I. Pignot-Paintrand, M. Vernet, P. Huber, Role of vascular endothelial-cadherin in vascular morphogenesis, *Development* 126 (1999) 2093-2102.
- [43] P. Carmeliet, M.G. Lampugnani, L. Moons, F. Breviario, V. Compernelle, F. Bono, G. Balconi, R. Spagnuolo, B. Oostuyse, M. Dewerchin, A. Zanetti, A. Angellilo, V. Mattot, D. Nuyens, E. Lutgens, F. Clotman, M.C. de Ruiter, A. Gittenberger-de Groot, R. Poelmann, F. Lupu, J.M. Herbert, D. Collen, E. Dejana, Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis, *Cell* 98 (1999) 147-157.
- [44] M. Corada, L. Zanetta, F. Orsenigo, F. Breviario, M.G. Lampugnani, S. Bernasconi, F. Liao, D.J. Hicklin, P. Bohlen, E. Dejana, A monoclonal antibody to vascular endothelial-cadherin inhibits tumor angiogenesis without side effects on endothelial permeability, *Blood* 100 (2002) 905-911.
- [45] F. Liao, J.F. Doody, J. Overholser, B. Finnerty, R. Bassi, Y. Wu, E. Dejana, P. Kussie, P. Bohlen, D.J. Hicklin, Selective targeting of angiogenic tumor vasculature by vascular endothelial-cadherin antibody inhibits tumor growth without affecting vascular permeability, *Cancer Res* 62 (2002) 2567-2575.
- [46] C. May, J.F. Doody, R. Abdullah, P. Balderes, X. Xu, C.P. Chen, Z. Zhu, L. Shapiro, P. Kussie, D.J. Hicklin, F. Liao, P. Bohlen, Identification of a transiently exposed VE-

- cadherin epitope that allows for specific targeting of an antibody to the tumor neovasculature, *Blood* 105 (2005) 4337-4344.
- [47] D. Salomon, O. Ayalon, R. Patel-King, R.O. Hynes, B. Geiger, Extrajunctional distribution of N-cadherin in cultured human endothelial cells, *J Cell Sci* 102 (1992) 7-17.
 - [48] D. Ivanov, M. Philippova, J. Antropova, F. Gubaeva, O. Iljinskaya, E. Tararak, V. Bochkov, P. Erne, T. Resink, V. Tkachuk, Expression of cell adhesion molecule T-cadherin in the human vasculature, *Histochemistry and cell biology* 115 (2001) 231-242.
 - [49] P. Telo, F. Breviario, P. Huber, C. Panzeri, E. Dejana, Identification of a novel cadherin (vascular endothelial cadherin-2) located at intercellular junctions in endothelial cells, *J Biol Chem* 273 (1998) 17565-17572.
 - [50] H. Gerhardt, H. Wolburg, C. Redies, N-cadherin mediates pericytic-endothelial interaction during brain angiogenesis in the chicken, *Dev Dyn* 218 (2000) 472-479.
 - [51] E. Tillet, D. Vittet, O. Feraud, R. Moore, R. Kemler, P. Huber, N-cadherin deficiency impairs pericyte recruitment, and not endothelial differentiation or sprouting, in embryonic stem cell-derived angiogenesis, *Exp Cell Res* 310 (2005) 392-400.
 - [52] D. Ivanov, M. Philippova, R. Allenspach, P. Erne, T. Resink, T-cadherin upregulation correlates with cell-cycle progression and promotes proliferation of vascular cells, *Cardiovasc Res* 64 (2004) 132-143.
 - [53] D. Ivanov, M. Philippova, V. Tkachuk, P. Erne, T. Resink, Cell adhesion molecule T-cadherin regulates vascular cell adhesion, phenotype and motility, *Exp Cell Res* 293 (2004) 207-218.
 - [54] M.B. Joshi, M. Philippova, D. Ivanov, R. Allenspach, P. Erne, T.J. Resink, T-cadherin protects endothelial cells from oxidative stress-induced apoptosis, *Faseb J* 19 (2005) 1737-1739.
 - [55] M. Philippova, D. Ivanov, V. Tkachuk, P. Erne, T.J. Resink, Polarisation of T-cadherin to the leading edge of migrating vascular cells in vitro: a function in vascular cell motility?, *Histochemistry and cell biology* 120 (2003) 353-360.
 - [56] A.M. Goodwin, P.A. D'Amore, Wnt signaling in the vasculature, *Angiogenesis* 5 (2002) 1-9.
 - [57] C.G. Eberhart, P. Argani, Wnt signaling in human development: beta-catenin nuclear translocation in fetal lung, kidney, placenta, capillaries, adrenal, and cartilage, *Pediatr Dev Pathol* 4 (2001) 351-357.
 - [58] C.G. Eberhart, T. Tihan, P.C. Burger, Nuclear localization and mutation of beta-catenin in medulloblastomas, *Journal of neuropathology and experimental neurology* 59 (2000) 333-337.
 - [59] W.M. Blankesteyn, M.E. van Gijn, Y.P. Essers-Janssen, M.J. Daemen, J.F. Smits, Beta-catenin, an inducer of uncontrolled cell proliferation and migration in malignancies, is localized in the cytoplasm of vascular endothelium during neovascularization after myocardial infarction, *Am J Pathol* 157 (2000) 877-883.
 - [60] S. Liebner, A. Cattelino, R. Gallini, N. Rudini, M. Iurlaro, S. Piccolo, E. Dejana, Beta-catenin is required for endothelial-mesenchymal transformation during heart cushion development in the mouse, *J Cell Biol* 166 (2004) 359-367.
 - [61] A. Cattelino, S. Liebner, R. Gallini, A. Zanetti, G. Balconi, A. Corsi, P. Bianco, H. Wolburg, R. Moore, B. Oreda, R. Kemler, E. Dejana, The conditional inactivation of the beta-catenin gene in endothelial cells causes a defective vascular pattern and increased vascular fragility, *J Cell Biol* 162 (2003) 1111-1122.
 - [62] M. Corada, F. Liao, M. Lindgren, M.G. Lampugnani, F. Breviario, R. Frank, W.A. Muller, D.J. Hicklin, P. Bohlen, E. Dejana, Monoclonal antibodies directed to

- different regions of vascular endothelial cadherin extracellular domain affect adhesion and clustering of the protein and modulate endothelial permeability, *Blood* 97 (2001) 1679-1684.
- [63] M. Corada, M. Mariotti, G. Thurston, K. Smith, R. Kunkel, M. Brockhaus, M.G. Lampugnani, I. Martin-Padura, A. Stoppacciaro, L. Ruco, D.M. McDonald, P.A. Ward, E. Dejana, Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo, *Proc Natl Acad Sci U S A* 96 (1999) 9815-9820.
 - [64] U. Gotsch, E. Borges, R. Bosse, E. Boggemeyer, M. Simon, H. Mossmann, D. Vestweber, VE-cadherin antibody accelerates neutrophil recruitment in vivo, *J Cell Sci* 110 (1997) 583-588.
 - [65] J.R. Allport, W.A. Muller, F.W. Luscinskas, Monocytes induce reversible focal changes in vascular endothelial cadherin complex during transendothelial migration under flow, *J Cell Biol* 148 (2000) 203-216.
 - [66] S.K. Shaw, P.S. Bamba, B.N. Perkins, F.W. Luscinskas, Real-time imaging of vascular endothelial-cadherin during leukocyte transmigration across endothelium, *J Immunol* 167 (2001) 2323-2330.
 - [67] W.H. Su, H.I. Chen, C.J. Jen, Differential movements of VE-cadherin and PECAM-1 during transmigration of polymorphonuclear leukocytes through human umbilical vein endothelium, *Blood* 100 (2002) 3597-3603.
 - [68] U. Eriksson, K. Alitalo, Structure, expression and receptor-binding properties of novel vascular endothelial growth factors, *Current topics in microbiology and immunology* 237 (1999) 41-57.
 - [69] S. Esser, M.G. Lampugnani, M. Corada, E. Dejana, W. Risau, Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells, *J Cell Sci* 111 (1998) 1853-1865.
 - [70] S. Weis, S. Shintani, A. Weber, R. Kirchmair, M. Wood, A. Cravens, H. McSharry, A. Iwakura, Y.S. Yoon, N. Himes, D. Burstein, J. Doukas, R. Soll, D. Losordo, D. Cheresh, Src blockade stabilizes a Flk/cadherin complex, reducing edema and tissue injury following myocardial infarction, *J Clin Invest* 113 (2004) 885-894.
 - [71] N. Lambeng, Y. Wallez, C. Rampon, F. Cand, G. Christe, D. Gulino-Debrac, I. Vilgrain, P. Huber, Vascular endothelial-cadherin tyrosine phosphorylation in angiogenic and quiescent adult tissues, *Circ Res* 96 (2005) 384-391.
 - [72] M.T. Chou, J. Wang, D.J. Fujita, Src kinase becomes preferentially associated with the VEGFR, KDR/Flk-1, following VEGF stimulation of vascular endothelial cells, *BMC Biochem* 3 (2002) 32.
 - [73] Y. Wallez, F. Cand, F. Cruzalegui, C. Wernstedt, S. Souchelnytskyi, I. Vilgrain, P. Huber, Src kinase phosphorylates vascular endothelial-cadherin in response to vascular endothelial growth factor: identification of tyrosine 685 as the unique target site, *Oncogene* 26 (2007) 1067-1077.
 - [74] U. Baumeister, R. Funke, K. Ebnet, H. Vorschmitt, S. Koch, D. Vestweber, Association of Csk to VE-cadherin and inhibition of cell proliferation, *Embo J* 24 (2005) 1686-1695.
 - [75] M.D. Potter, S. Barbero, D.A. Cheresh, Tyrosine phosphorylation of VE-cadherin prevents binding of p120- and {beta}-catenin and maintains the cellular mesenchymal state, *J Biol Chem* 280 (2005) 31906-31912.
 - [76] J. Gavard, J.S. Gutkind, VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin, *Nature cell biology* 8 (2006) 1223-1234.

- [77] P. Andriopoulou, P. Navarro, A. Zanetti, M.G. Lampugnani, E. Dejana, Histamine induces tyrosine phosphorylation of endothelial cell-to-cell adherens junctions, *Arterioscler Thromb Vasc Biol* 19 (1999) 2286-2297.
- [78] D.M. Shasby, D.R. Ries, S.S. Shasby, M.C. Winter, Histamine stimulates phosphorylation of adherens junction proteins and alters their link to vimentin, *Am J Physiol Lung Cell Mol Physiol* 282 (2002) L1330-1338.
- [79] F.E. Nwariaku, Z. Liu, X. Zhu, R.H. Turnage, G.A. Sarosi, L.S. Terada, Tyrosine phosphorylation of vascular endothelial cadherin and the regulation of microvascular permeability, *Surgery* 132 (2002) 180-185.
- [80] D.J. Angelini, S.W. Hyun, D.N. Grigoryev, P. Garg, P. Gong, I.S. Singh, A. Passaniti, J.D. Hasday, S.E. Goldblum, TNF-alpha increases tyrosine phosphorylation of vascular endothelial cadherin and opens the paracellular pathway through fyn activation in human lung endothelia, *Am J Physiol Lung Cell Mol Physiol* 291 (2006) L1232-1245.
- [81] J.H. Tinsley, M.H. Wu, W. Ma, A.C. Taulman, S.Y. Yuan, Activated neutrophils induce hyperpermeability and phosphorylation of adherens junction proteins in coronary venular endothelial cells, *J Biol Chem* 274 (1999) 24930-24934.
- [82] H. Hudry-Clergeon, D. Stengel, E. Ninio, I. Vilgrain, Platelet-activating factor increases VE-cadherin tyrosine phosphorylation in mouse endothelial cells and its association with the PtdIns3'-kinase, *Faseb J* 19 (2005) 512-520.
- [83] Y. Wang, G. Jin, H. Miao, J.Y. Li, S. Usami, S. Chien, Integrins regulate VE-cadherin and catenins: dependence of this regulation on Src, but not on Ras, *Proc Natl Acad Sci U S A* 103 (2006) 1774-1779.
- [84] M.G. Lampugnani, A. Zanetti, M. Corada, T. Takahashi, G. Balconi, F. Breviario, F. Orsenigo, A. Cattelino, R. Kemler, T.O. Daniel, E. Dejana, Contact inhibition of VEGF-induced proliferation requires vascular endothelial cadherin, beta-catenin, and the phosphatase DEP-1/CD148, *J Cell Biol* 161 (2003) 793-804.
- [85] R. Nawroth, G. Poell, A. Ranft, S. Kloep, U. Samulowitz, G. Fachinger, M. Golding, D.T. Shima, U. Deutsch, D. Vestweber, VE-PTP and VE-cadherin ectodomains interact to facilitate regulation of phosphorylation and cell contacts, *Embo J* 24 (2005) 3158.
- [86] C. Bianchi, F.W. Sellke, R.L. Del Vecchio, N.K. Tonks, B.G. Neel, Receptor-type protein-tyrosine phosphatase mu is expressed in specific vascular endothelial beds in vivo, *Exp Cell Res* 248 (1999) 329-338.
- [87] X.F. Sui, T.D. Kiser, S.W. Hyun, D.J. Angelini, R.L. Del Vecchio, B.A. Young, J.D. Hasday, L.H. Romer, A. Passaniti, N.K. Tonks, S.E. Goldblum, Receptor protein tyrosine phosphatase micro regulates the paracellular pathway in human lung microvascular endothelia, *Am J Pathol* 166 (2005) 1247-1258.
- [88] J.A. Ukropec, M.K. Hollinger, S.M. Salva, M.J. Woolkalis, SHP2 association with VE-cadherin complexes in human endothelial cells is regulated by thrombin, *J Biol Chem* 275 (2000) 5983-5986.
- [89] D. Carden, F. Xiao, C. Moak, B.H. Willis, S. Robinson-Jackson, S. Alexander, Neutrophil elastase promotes lung microvascular injury and proteolysis of endothelial cadherins, *Am J Physiol* 275 (1998) H385-392.
- [90] B. Hermant, S. Bibert, E. Concord, B. Dublet, M. Weidenhaupt, T. Vernet, D. Gulino-Debrac, Identification of proteases involved in the proteolysis of vascular endothelium cadherin during neutrophil transmigration, *J Biol Chem* 278 (2003) 14002-14012.
- [91] J.R. Allport, Y.C. Lim, J.M. Shipley, R.M. Senior, S.D. Shapiro, N. Matsuyoshi, D. Vestweber, F.W. Luscinskas, Neutrophils from MMP-9- or neutrophil elastase-

- deficient mice show no defect in transendothelial migration under flow in vitro, *Journal of leukocyte biology* 71 (2002) 821-828.
- [92] B. Herren, B. Levkau, E.W. Raines, R. Ross, Cleavage of beta-catenin and plakoglobin and shedding of VE-cadherin during endothelial apoptosis: evidence for a role for caspases and metalloproteinases, *Mol Biol Cell* 9 (1998) 1589-1601.
 - [93] Y. Ichikawa, T. Ishikawa, N. Momiyama, M. Kamiyama, H. Sakurada, R. Matsuyama, S. Hasegawa, T. Chishima, Y. Hamaguchi, S. Fujii, S. Saito, K. Kubota, S. Hasegawa, H. Ike, S. Oki, H. Shimada, Matrilysin (MMP-7) degrades VE-cadherin and accelerates accumulation of beta-catenin in the nucleus of human umbilical vein endothelial cells, *Oncology reports* 15 (2006) 311-315.
 - [94] N. Luplertlop, D. Misse, D. Bray, V. Deleuze, J.P. Gonzalez, V. Leardkamolkarn, H. Yssel, F. Veas, Dengue-virus-infected dendritic cells trigger vascular leakage through metalloproteinase overproduction, *EMBO reports* 7 (2006) 1176-1181.
 - [95] D. Navaratna, P.G. McGuire, G. Menicucci, A. Das, Proteolytic Degradation of VE-Cadherin Alters the Blood-Retinal Barrier in Diabetes, *Diabetes* (2007).
 - [96] W.B. Wu, T.F. Huang, Activation of MMP-2, cleavage of matrix proteins, and adherens junctions during a snake venom metalloproteinase-induced endothelial cell apoptosis, *Exp Cell Res* 288 (2003) 143-157.
 - [97] K. Xiao, D.F. Allison, K.M. Buckley, M.D. Kottke, P.A. Vincent, V. Faundez, A.P. Kowalczyk, Cellular levels of p120 catenin function as a set point for cadherin expression levels in microvascular endothelial cells, *J Cell Biol* 163 (2003) 535-545.
 - [98] K. Xiao, D.F. Allison, M.D. Kottke, S. Summers, G.P. Sorescu, V. Faundez, A.P. Kowalczyk, Mechanisms of VE-cadherin processing and degradation in microvascular endothelial cells, *J Biol Chem* 278 (2003) 19199-19208.
 - [99] S. Iyer, D.M. Ferreri, N.C. DeCocco, F.L. Minnear, P.A. Vincent, VE-cadherin-p120 interaction is required for maintenance of endothelial barrier function, *Am J Physiol Lung Cell Mol Physiol* 286 (2004) L1143-1153.
 - [100] K. Xiao, J. Garner, K.M. Buckley, P.A. Vincent, C.M. Chiasson, E. Dejana, V. Faundez, A.P. Kowalczyk, p120-Catenin regulates clathrin-dependent endocytosis of VE-cadherin, *Mol Biol Cell* 16 (2005) 5141-5151.
 - [101] L. Caveda, I. Martin-Padura, P. Navarro, F. Breviario, M. Corada, D. Gulino, M.G. Lampugnani, E. Dejana, Inhibition of cultured cell growth by vascular endothelial cadherin (cadherin-5/VE-cadherin), *J Clin Invest* 98 (1996) 886-893.
 - [102] M. Pierce, C. Wang, M. Stump, A. Kamb, Overexpression of the beta-catenin binding domain of cadherin selectively kills colorectal cancer cells, *International journal of cancer* 107 (2003) 229-237.
 - [103] M.G. Lampugnani, F. Orsenigo, M.C. Gagliani, C. Tacchetti, E. Dejana, Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments, *J Cell Biol* 174 (2006) 593-604.
 - [104] A. Zanetti, M.G. Lampugnani, G. Balconi, F. Breviario, M. Corada, L. Lanfrancone, E. Dejana, Vascular endothelial growth factor induces SHC association with vascular endothelial cadherin: a potential feedback mechanism to control vascular endothelial growth factor receptor-2 signaling, *Arterioscler Thromb Vasc Biol* 22 (2002) 617-622.
 - [105] G. Pelicci, L. Lanfrancone, F. Grignani, J. McGlade, F. Cavallo, G. Forni, I. Nicoletti, F. Grignani, T. Pawson, P.G. Pelicci, A novel transforming protein (SHC) with an SH2 domain is implicated in mitogenic signal transduction, *Cell* 70 (1992) 93-104.
 - [106] S.C. Harrison, Variation on an Src-like theme, *Cell* 112 (2003) 737-740.
 - [107] L.J. Duan, A. Imamoto, G.H. Fong, Dual roles of the C-terminal Src kinase (Csk) during developmental vascularization, *Blood* 103 (2004) 1370-1372.

- [108] C.M. Nelson, C.S. Chen, VE-cadherin simultaneously stimulates and inhibits cell proliferation by altering cytoskeletal structure and tension, *J Cell Sci* 116 (2003) 3571-3581.
- [109] R. Spagnuolo, M. Corada, F. Orsenigo, L. Zanetta, U. Deuschle, P. Sandy, C. Schneider, C.J. Drake, F. Breviario, E. Dejana, Gas1 is induced by VE-cadherin and vascular endothelial growth factor and inhibits endothelial cell apoptosis, *Blood* 103 (2004) 3005-3012.
- [110] H.J. Schnittler, B. Puschel, D. Drenckhahn, Role of cadherins and plakoglobin in interendothelial adhesion under resting conditions and shear stress, *Am J Physiol* 273 (1997) H2396-2405.
- [111] A. Shay-Salit, M. Shushy, E. Wolfowitz, H. Yahav, F. Breviario, E. Dejana, N. Resnick, VEGF receptor 2 and the adherens junction as a mechanical transducer in vascular endothelial cells, *Proc Natl Acad Sci U S A* 99 (2002) 9462-9467.
- [112] E. Tzima, M. Irani-Tehrani, W.B. Kiosses, E. Dejana, D.A. Schultz, B. Engelhardt, G. Cao, H. DeLisser, M.A. Schwartz, A mechanosensory complex that mediates the endothelial cell response to fluid shear stress, *Nature* 437 (2005) 426-431.
- [113] J. Waschke, W. Baumgartner, R.H. Adamson, M. Zeng, K. Aktories, H. Barth, C. Wilde, F.E. Curry, D. Drenckhahn, Requirement of Rac activity for maintenance of capillary endothelial barrier properties, *American journal of physiology* 286 (2004) H394-401.
- [114] J. Waschke, D. Drenckhahn, R.H. Adamson, F.E. Curry, Role of adhesion and contraction in Rac 1-regulated endothelial barrier function in vivo and in vitro, *American journal of physiology* 287 (2004) H704-711.
- [115] B. Wojciak-Stothard, L.Y. Tsang, S.G. Haworth, Rac and Rho play opposing roles in the regulation of hypoxia/reoxygenation-induced permeability changes in pulmonary artery endothelial cells, *Am J Physiol Lung Cell Mol Physiol* 288 (2005) L749-760.
- [116] Y. Liu, D.R. Senger, Matrix-specific activation of Src and Rho initiates capillary morphogenesis of endothelial cells, *Faseb J* 18 (2004) 457-468.
- [117] J. Seebach, H.J. Madler, B. Wojciak-Stothard, H.J. Schnittler, Tyrosine phosphorylation and the small GTPase rac cross-talk in regulation of endothelial barrier function, *Thrombosis and haemostasis* 94 (2005) 620-629.
- [118] S. van Wetering, J.D. van Buul, S. Quik, F.P. Mul, E.C. Anthony, J.P. ten Klooster, J.G. Collard, P.L. Hordijk, Reactive oxygen species mediate Rac-induced loss of cell-cell adhesion in primary human endothelial cells, *J Cell Sci* 115 (2002) 1837-1846.
- [119] J.D. van Buul, E.C. Anthony, M. Fernandez-Borja, K. Burridge, P.L. Hordijk, Proline-rich tyrosine kinase 2 (Pyk2) mediates vascular endothelial-cadherin-based cell-cell adhesion by regulating beta-catenin tyrosine phosphorylation, *J Biol Chem* 280 (2005) 21129-21136.
- [120] M.G. Lampugnani, A. Zanetti, F. Breviario, G. Balconi, F. Orsenigo, M. Corada, R. Spagnuolo, M. Betson, V. Braga, E. Dejana, VE-cadherin regulates endothelial actin activating Rac and increasing membrane association of Tiam, *Mol Biol Cell* 13 (2002) 1175-1189.
- [121] C.M. Nelson, D.M. Pirone, J.L. Tan, C.S. Chen, Vascular endothelial-cadherin regulates cytoskeletal tension, cell spreading, and focal adhesions by stimulating RhoA, *Mol Biol Cell* 15 (2004) 2943-2953.
- [122] P. Kouklis, M. Konstantoulaki, S. Vogel, M. Broman, A.B. Malik, Cdc42 regulates the restoration of endothelial barrier function, *Circ Res* 94 (2004) 159-166.
- [123] M.T. Broman, P. Kouklis, X. Gao, R. Ramchandran, R.F. Neamu, R.D. Minshall, A.B. Malik, Cdc42 regulates adherens junction stability and endothelial permeability by

- inducing alpha-catenin interaction with the vascular endothelial cadherin complex, *Circ Res* 98 (2006) 73-80.
- [124] P. Kouklis, M. Konstantoulaki, A.B. Malik, VE-cadherin-induced Cdc42 signaling regulates formation of membrane protrusions in endothelial cells, *J Biol Chem* 278 (2003) 16230-16236.
 - [125] J. Martinez, A. Ferber, T.L. Bach, C.H. Yaen, Interaction of fibrin with VE-cadherin, *Ann N Y Acad Sci* 936 (2001) 386-405.
 - [126] T.J. Stelzner, J.V. Weil, R.F. O'Brien, Role of cyclic adenosine monophosphate in the induction of endothelial barrier properties, *Journal of cellular physiology* 139 (1989) 157-166.
 - [127] P.J. Farmer, S.G. Bernier, A. Lepage, G. Guillemette, D. Regoli, P. Sirois, Permeability of endothelial monolayers to albumin is increased by bradykinin and inhibited by prostaglandins, *Am J Physiol Lung Cell Mol Physiol* 280 (2001) L732-738.
 - [128] S. Fukuhara, A. Sakurai, H. Sano, A. Yamagishi, S. Somekawa, N. Takakura, Y. Saito, K. Kangawa, N. Mochizuki, Cyclic AMP potentiates vascular endothelial cadherin-mediated cell-cell contact to enhance endothelial barrier function through an Epac-Rap1 signaling pathway, *Mol Cell Biol* 25 (2005) 136-146.
 - [129] M.R. Kooistra, M. Corada, E. Dejana, J.L. Bos, Epac1 regulates integrity of endothelial cell junctions through VE-cadherin, *FEBS letters* 579 (2005) 4966-4972.
 - [130] A. Sakurai, S. Fukuhara, A. Yamagishi, K. Sako, Y. Kamioka, M. Masuda, Y. Nakaoka, N. Mochizuki, MAGI-1 is required for Rap1 activation upon cell-cell contact and for enhancement of vascular endothelial cadherin-mediated cell adhesion, *Mol Biol Cell* 17 (2006) 966-976.
 - [131] W.C. Aird, Phenotypic heterogeneity of the endothelium: II. Representative vascular beds, *Circ Res* 100 (2007) 174-190.
 - [132] W.C. Aird, Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms, *Circ Res* 100 (2007) 158-173.
 - [133] J.H. Kim, J.H. Kim, J.A. Park, S.W. Lee, W.J. Kim, Y.S. Yu, K.W. Kim, Blood-neural barrier: intercellular communication at glio-vascular interface, *Journal of biochemistry and molecular biology* 39 (2006) 339-345.
 - [134] C.H. Lai, K.H. Kuo, The critical component to establish in vitro BBB model: Pericyte, *Brain research* 50 (2005) 258-265.
 - [135] M. Furuse, S. Tsukita, Claudins in occluding junctions of humans and flies, *Trends in cell biology* 16 (2006) 181-188.
 - [136] S. Hori, S. Ohtsuki, K. Hosoya, E. Nakashima, T. Terasaki, A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro, *Journal of neurochemistry* 89 (2004) 503-513.
 - [137] S. Dohgu, F. Takata, A. Yamauchi, S. Nakagawa, T. Egawa, M. Naito, T. Tsuruo, Y. Sawada, M. Niwa, Y. Kataoka, Brain pericytes contribute to the induction and up-regulation of blood-brain barrier functions through transforming growth factor-beta production, *Brain Res* 1038 (2005) 208-215.
 - [138] S. Dohgu, A. Yamauchi, F. Takata, M. Naito, T. Tsuruo, S. Higuchi, Y. Sawada, Y. Kataoka, Transforming growth factor-beta1 upregulates the tight junction and P-glycoprotein of brain microvascular endothelial cells, *Cellular and molecular neurobiology* 24 (2004) 491-497.
 - [139] S. Aijaz, M.S. Balda, K. Matter, Tight junctions: molecular architecture and function, *International review of cytology* 248 (2006) 261-298.

- [140] K. Morita, H. Sasaki, K. Furuse, M. Furuse, S. Tsukita, Y. Miyachi, Expression of claudin-5 in dermal vascular endothelia, *Experimental dermatology* 12 (2003) 289-295.
- [141] T. Nitta, M. Hata, S. Gotoh, Y. Seo, H. Sasaki, N. Hashimoto, M. Furuse, S. Tsukita, Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice, *J Cell Biol* 161 (2003) 653-660.
- [142] J.C. Challier, G. Dubernard, M. Galtier, T. Bintein, C. Vervelle, D. Raison, M.J. Espie, S. Uzan, Junctions and adhesion molecules in first trimester and term human placentas, *Cellular and molecular biology (Noisy-le-Grand, France)* 51 Suppl (2005) OL713-722.
- [143] S. Lievano, L. Alarcon, B. Chavez-Munguia, L. Gonzalez-Mariscal, Endothelia of term human placentae display diminished expression of tight junction proteins during preeclampsia, *Cell and tissue research* 324 (2006) 433-448.
- [144] H. Wolburg, K. Wolburg-Buchholz, J. Kraus, G. Rascher-Eggstein, S. Liebner, S. Hamm, F. Duffner, E.H. Grote, W. Risau, B. Engelhardt, Localization of claudin-3 in tight junctions of the blood-brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme, *Acta neuropathologica* 105 (2003) 586-592.
- [145] B.E. Enerson, L.R. Drewes, The rat blood-brain barrier transcriptome, *J Cereb Blood Flow Metab* 26 (2006) 959-973.
- [146] S. Liebner, A. Fischmann, G. Rascher, F. Duffner, E.H. Grote, H. Kalbacher, H. Wolburg, Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme, *Acta neuropathologica* 100 (2000) 323-331.
- [147] G. Bazzoni, Endothelial tight junctions: permeable barriers of the vessel wall, *Thrombosis and haemostasis* 95 (2006) 36-42.
- [148] D.C. Davies, Blood-brain barrier breakdown in septic encephalopathy and brain tumours, *Journal of anatomy* 200 (2002) 639-646.
- [149] N.S. Harhaj, D.A. Antonetti, Regulation of tight junctions and loss of barrier function in pathophysiology, *Int J Biochem Cell Biol* 36 (2004) 1206-1237.
- [150] A. MacIntyre, C.J. Hammond, C.S. Little, D.M. Appelt, B.J. Balin, Chlamydia pneumoniae infection alters the junctional complex proteins of human brain microvascular endothelial cells, *FEMS microbiology letters* 217 (2002) 167-172.
- [151] N. Sawada, M. Murata, K. Kikuchi, M. Osanai, H. Tobioka, T. Kojima, H. Chiba, Tight junctions and human diseases, *Med Electron Microsc* 36 (2003) 147-156.
- [152] B.T. Hawkins, T.J. Abbruscato, R.D. Egleton, R.C. Brown, J.D. Huber, C.R. Campos, T.P. Davis, Nicotine increases in vivo blood-brain barrier permeability and alters cerebral microvascular tight junction protein distribution, *Brain Res* 1027 (2004) 48-58.
- [153] D.A. Antonetti, A.J. Barber, S. Khin, E. Lieth, J.M. Tarbell, T.W. Gardner, Vascular permeability in experimental diabetes is associated with reduced endothelial occludin content: vascular endothelial growth factor decreases occludin in retinal endothelial cells. Penn State Retina Research Group, *Diabetes* 47 (1998) 1953-1959.
- [154] K.K. Erickson, J.M. Sundstrom, D.A. Antonetti, Vascular permeability in ocular disease and the role of tight junctions, *Angiogenesis* 10 (2007) 103-117.
- [155] J.D. Huber, R.D. Egleton, T.P. Davis, Molecular physiology and pathophysiology of tight junctions in the blood-brain barrier, *Trends in neurosciences* 24 (2001) 719-725.
- [156] P. Vajkoczy, M.D. Menger, Vascular microenvironment in gliomas, *Journal of neuro-oncology* 50 (2000) 99-108.

- [157] R.C. Brown, T.P. Davis, Calcium modulation of adherens and tight junction function: a potential mechanism for blood-brain barrier disruption after stroke, *Stroke; a journal of cerebral circulation* 33 (2002) 1706-1711.
- [158] R.C. Brown, T.P. Davis, Hypoxia/aglycemia alters expression of occludin and actin in brain endothelial cells, *Biochem Biophys Res Commun* 327 (2005) 1114-1123.
- [159] M. Saitou, M. Furuse, H. Sasaki, J.D. Schulzke, M. Fromm, H. Takano, T. Noda, S. Tsukita, Complex phenotype of mice lacking occludin, a component of tight junction strands, *Mol Biol Cell* 11 (2000) 4131-4142.
- [160] M.A. Behzadian, L.J. Windsor, N. Ghaly, G. Liou, N.T. Tsai, R.B. Caldwell, VEGF-induced paracellular permeability in cultured endothelial cells involves urokinase and its receptor, *Faseb J* 17 (2003) 752-754.
- [161] A. Reijerkerk, G. Kooij, S.M. van der Pol, S. Khazen, C.D. Dijkstra, H.E. de Vries, Diapedesis of monocytes is associated with MMP-mediated occludin disappearance in brain endothelial cells, *Faseb J* 20 (2006) 2550-2552.
- [162] M. Wachtel, K. Frei, E. Ehler, A. Fontana, K. Winterhalter, S.M. Gloor, Occludin proteolysis and increased permeability in endothelial cells through tyrosine phosphatase inhibition, *J Cell Sci* 112 (Pt 23) (1999) 4347-4356.
- [163] D.A. Antonetti, A.J. Barber, L.A. Hollinger, E.B. Wolpert, T.W. Gardner, Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors, *J Biol Chem* 274 (1999) 23463-23467.
- [164] N.S. Harhaj, E.A. Felinski, E.B. Wolpert, J.M. Sundstrom, T.W. Gardner, D.A. Antonetti, VEGF activation of protein kinase C stimulates occludin phosphorylation and contributes to endothelial permeability, *Investigative ophthalmology & visual science* 47 (2006) 5106-5115.
- [165] L. DeMaio, Y.S. Chang, T.W. Gardner, J.M. Tarbell, D.A. Antonetti, Shear stress regulates occludin content and phosphorylation, *American journal of physiology* 281 (2001) H105-113.
- [166] L. DeMaio, M. Rouhanizadeh, S. Reddy, A. Sevanian, J. Hwang, T.K. Hsiai, Oxidized phospholipids mediate occludin expression and phosphorylation in vascular endothelial cells, *American journal of physiology* 290 (2006) H674-683.
- [167] T. Hirase, S. Kawashima, E.Y. Wong, T. Ueyama, Y. Rikitake, S. Tsukita, M. Yokoyama, J.M. Staddon, Regulation of tight junction permeability and occludin phosphorylation by RhoA-p160ROCK-dependent and -independent mechanisms, *J Biol Chem* 276 (2001) 10423-10431.
- [168] S.M. Stamatovic, O.B. Dimitrijevic, R.F. Keep, A.V. Andjelkovic, Protein kinase Calpha-RhoA cross-talk in CCL2-induced alterations in brain endothelial permeability, *J Biol Chem* 281 (2006) 8379-8388.
- [169] K. Wosik, R. Cayrol, A. Dodelet-Devillers, F. Berthelet, M. Bernard, R. Moumdjian, A. Bouthillier, T.L. Reudelhuber, A. Prat, Angiotensin II controls occludin function and is required for blood brain barrier maintenance: relevance to multiple sclerosis, *J Neurosci* 27 (2007) 9032-9042.
- [170] T. Kago, N. Takagi, I. Date, Y. Takenaga, K. Takagi, S. Takeo, Cerebral ischemia enhances tyrosine phosphorylation of occludin in brain capillaries, *Biochem Biophys Res Commun* 339 (2006) 1197-1203.
- [171] D.A. Antonetti, E.B. Wolpert, L. DeMaio, N.S. Harhaj, R.C. Scaduto, Jr., Hydrocortisone decreases retinal endothelial cell water and solute flux coincident with increased content and decreased phosphorylation of occludin, *Journal of neurochemistry* 80 (2002) 667-677.

- [172] M.S. Balda, J.M. Anderson, Two classes of tight junctions are revealed by ZO-1 isoforms, *Am J Physiol* 264 (1993) C918-924.
- [173] E. Willott, M.S. Balda, M. Heintzelman, B. Jameson, J.M. Anderson, Localization and differential expression of two isoforms of the tight junction protein ZO-1, *Am J Physiol* 262 (1992) C1119-1124.
- [174] H. Kurihara, J.M. Anderson, M.G. Farquhar, Diversity among tight junctions in rat kidney: glomerular slit diaphragms and endothelial junctions express only one isoform of the tight junction protein ZO-1, *Proc Natl Acad Sci U S A* 89 (1992) 7075-7079.
- [175] B.H. Keon, S. Schafer, C. Kuhn, C. Grund, W.W. Franke, Symplekin, a novel type of tight junction plaque protein, *J Cell Biol* 134 (1996) 1003-1018.
- [176] M. Aurrand-Lions, C. Johnson-Leger, C. Wong, L. Du Pasquier, B.A. Imhof, Heterogeneity of endothelial junctions is reflected by differential expression and specific subcellular localization of the three JAM family members, *Blood* 98 (2001) 3699-3707.
- [177] L.A. Williams, I. Martin-Padura, E. Dejana, N. Hogg, D.L. Simmons, Identification and characterisation of human Junctional Adhesion Molecule (JAM), *Molecular immunology* 36 (1999) 1175-1188.
- [178] C. Ody, S. Jungblut-Ruault, D. Cossali, M. Barnet, M. Aurrand-Lions, B.A. Imhof, T. Matthes, Junctional adhesion molecule C (JAM-C) distinguishes CD27+ germinal center B lymphocytes from non-germinal center cells and constitutes a new diagnostic tool for B-cell malignancies, *Leukemia* 21 (2007) 1285-1293.
- [179] A.P. Morris, A. Tawil, Z. Berkova, L. Wible, C.W. Smith, S.A. Cunningham, Junctional Adhesion Molecules (JAMs) are differentially expressed in fibroblasts and co-localize with ZO-1 to adherens-like junctions, *Cell communication & adhesion* 13 (2006) 233-247.
- [180] D. Palmeri, A. van Zante, C.C. Huang, S. Hemmerich, S.D. Rosen, Vascular endothelial junction-associated molecule, a novel member of the immunoglobulin superfamily, is localized to intercellular boundaries of endothelial cells, *J Biol Chem* 275 (2000) 19139-19145.
- [181] T.W. Liang, R.A. DeMarco, R.J. Mrsny, A. Gurney, A. Gray, J. Hooley, H.L. Aaron, A. Huang, T. Klassen, D.B. Tumas, S. Fong, Characterization of huJAM: evidence for involvement in cell-cell contact and tight junction regulation, *Am J Physiol Cell Physiol* 279 (2000) C1733-1743.
- [182] Y. Liu, A. Nusrat, F.J. Schnell, T.A. Reaves, S. Walsh, M. Pochet, C.A. Parkos, Human junction adhesion molecule regulates tight junction resealing in epithelia, *J Cell Sci* 113 (Pt 13) (2000) 2363-2374.
- [183] E. Dejana, R. Spagnuolo, G. Bazzoni, Interendothelial junctions and their role in the control of angiogenesis, vascular permeability and leukocyte transmigration, *Thrombosis and haemostasis* 86 (2001) 308-315.
- [184] H. Ozaki, K. Ishii, H. Horiuchi, H. Arai, T. Kawamoto, K. Okawa, A. Iwamatsu, T. Kita, Cutting edge: combined treatment of TNF-alpha and IFN-gamma causes redistribution of junctional adhesion molecule in human endothelial cells, *J Immunol* 163 (1999) 553-557.
- [185] S. Nourshargh, F. Krombach, E. Dejana, The role of JAM-A and PECAM-1 in modulating leukocyte infiltration in inflamed and ischemic tissues, *Journal of leukocyte biology* 80 (2006) 714-718.
- [186] G. Ostermann, K.S. Weber, A. Zerneck, A. Schroder, C. Weber, JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes, *Nature immunology* 3 (2002) 151-158.

- [187] M.R. Cera, A. Del Prete, A. Vecchi, M. Corada, I. Martin-Padura, T. Motoike, P. Tonetti, G. Bazzoni, W. Vermi, F. Gentili, S. Bernasconi, T.N. Sato, A. Mantovani, E. Dejana, Increased DC trafficking to lymph nodes and contact hypersensitivity in junctional adhesion molecule-A-deficient mice, *J Clin Invest* 114 (2004) 729-738.
- [188] V.G. Cooke, M.U. Naik, U.P. Naik, Fibroblast growth factor-2 failed to induce angiogenesis in junctional adhesion molecule-A-deficient mice, *Arterioscler Thromb Vasc Biol* 26 (2006) 2005-2011.
- [189] M. Corada, S. Chimenti, M.R. Cera, M. Vinci, M. Salio, F. Fiordaliso, N. De Angelis, A. Villa, M. Bossi, L.I. Staszewsky, A. Vecchi, D. Parazzoli, T. Motoike, R. Latini, E. Dejana, Junctional adhesion molecule-A-deficient polymorphonuclear cells show reduced diapedesis in peritonitis and heart ischemia-reperfusion injury, *Proc Natl Acad Sci U S A* 102 (2005) 10634-10639.
- [190] A. Khandoga, J.S. Kessler, H. Meissner, M. Hanschen, M. Corada, T. Motoike, G. Enders, E. Dejana, F. Krombach, Junctional adhesion molecule-A deficiency increases hepatic ischemia-reperfusion injury despite reduction of neutrophil transendothelial migration, *Blood* 106 (2005) 725-733.
- [191] M. Aurrand-Lions, L. Duncan, C. Ballestrem, B.A. Imhof, JAM-2, a novel immunoglobulin superfamily molecule, expressed by endothelial and lymphatic cells, *J Biol Chem* 276 (2001) 2733-2741.
- [192] C. Lamagna, P. Meda, G. Mandicourt, J. Brown, R.J. Gilbert, E.Y. Jones, F. Kiefer, P. Ruga, B.A. Imhof, M. Aurrand-Lions, Dual interaction of JAM-C with JAM-B and alpha(M)beta2 integrin: function in junctional complexes and leukocyte adhesion, *Mol Biol Cell* 16 (2005) 4992-5003.
- [193] V.V. Orlova, M. Economopoulou, F. Lupu, S. Santoso, T. Chavakis, Junctional adhesion molecule-C regulates vascular endothelial permeability by modulating VE-cadherin-mediated cell-cell contacts, *The Journal of experimental medicine* 203 (2006) 2703-2714.
- [194] M. Sircar, P.F. Bradfield, M. Aurrand-Lions, R.J. Fish, P. Alcaide, L. Yang, G. Newton, D. Lamont, S. Sehrawat, T. Mayadas, T.W. Liang, C.A. Parkos, B.A. Imhof, F.W. Luscinskas, Neutrophil Transmigration under Shear Flow Conditions In Vitro Is Junctional Adhesion Molecule-C Independent, *J Immunol* 178 (2007) 5879-5887.
- [195] C. Fuse, Y. Ishida, T. Hikita, T. Asai, N. Oku, Junctional adhesion molecule-C promotes metastatic potential of HT1080 human fibrosarcoma, *J Biol Chem* 282 (2007) 8276-8283.
- [196] S. Santoso, V.V. Orlova, K. Song, U.J. Sachs, C.L. Andrei-Selmer, T. Chavakis, The homophilic binding of junctional adhesion molecule-C mediates tumor cell-endothelial cell interactions, *J Biol Chem* 280 (2005) 36326-36333.
- [197] G. Mandicourt, S. Iden, K. Ebnet, M. Aurrand-Lions, B.A. Imhof, JAM-C regulates tight junctions and integrin-mediated cell adhesion and migration, *J Biol Chem* 282 (2007) 1830-1837.
- [198] B. Imhof, C. Zimmerli, G. Gliki, D. Ducrest-Gay, P. Juillard, P. Hammel, R. Adams, M. Aurrand-Lions, Pulmonary dysfunction and impaired granulocyte homeostasis result in poor survival of Jam-C-deficient mice, *The Journal of pathology* 212 (2007) 198-208.
- [199] F. Wegmann, K. Ebnet, L. Du Pasquier, D. Vestweber, S. Butz, Endothelial adhesion molecule ESAM binds directly to the multidomain adaptor MAGI-1 and recruits it to cell contacts, *Exp Cell Res* 300 (2004) 121-133.
- [200] T. Ishida, R.K. Kundu, E. Yang, K. Hirata, Y.D. Ho, T. Quertermous, Targeted disruption of endothelial cell-selective adhesion molecule inhibits angiogenic processes in vitro and in vivo, *J Biol Chem* 278 (2003) 34598-34604.

- [201] F. Wegmann, B. Petri, A.G. Khandoga, C. Moser, A. Khandoga, S. Volkery, H. Li, I. Nasdala, O. Brandau, R. Fassler, S. Butz, F. Krombach, D. Vestweber, ESAM supports neutrophil extravasation, activation of Rho, and VEGF-induced vascular permeability, *The Journal of experimental medicine* 203 (2006) 1671-1677.
- [202] K. Irie, K. Shimizu, T. Sakisaka, W. Ikeda, Y. Takai, Roles and modes of action of nectins in cell-cell adhesion, *Seminars in cell & developmental biology* 15 (2004) 643-656.
- [203] H. Nakanishi, Y. Takai, Roles of nectins in cell adhesion, migration and polarization, *Biol Chem* 385 (2004) 885-892.
- [204] M. Lopez, M. Aoubala, F. Jordier, D. Isnardon, S. Gomez, P. Dubreuil, The human poliovirus receptor related 2 protein is a new hematopoietic/endothelial homophilic adhesion molecule, *Blood* 92 (1998) 4602-4611.

FIGURE LEGENDS

Fig. 1: Electron micrograph showing an endothelial junction in mouse aorta

The intercellular cleft (between arrowheads) is lined by two endothelial (E) strands and extends from basal lamina to lumen (L).

Fig. 2: Adhesive proteins within the interendothelial cleft

Claudins, occluding, JAMs and ESAM are located in TJs, VE-cadherin in AJs and connexins in gap junctions. Nectin was detected in both AJs and TJs, while PECAM, Pcdh12, CD99 and S-endo-1 are outside of these structures. TJs and AJs are both linked to the actin cytoskeleton and VE-cadherin may also be associated with vimentin filaments in some vascular beds.

Fig. 3: Model of hexameric assembly of VE-cadherin extracellular domains deduced from biochemical analyses and electron-microscopy

Model includes VE-cadherin ectodomains (EC) 1-4. Left: model with the VE-cadherin dimers represented in violet, light-green and light-blue. Right: The fit of the VE-cadherin model in the reconstructed hexamer. EC1 (red), EC2 (green), EC3 (cyan) and EC4 (blue) are coloured as indicated. Reproduced from [27]

Fig. 4: Adherens junctions and complexus adherentes in endothelial cells

In AJs, VE-cadherin directly interacts at a membrane-distal site with α - and β -catenins and at a juxtramembrane site with p120 and p0071. The exact molecular link between the VE-cadherin-catenin complex and the actin filaments is still obscure. The nectins are linked to the actin cytoskeleton via afadin. In complexus adherens, VE-cadherin associates with vimentin filaments via plakoglobin/desmoplakin or p0071.

Fig. 5: VEGF-induced VE-cadherin phosphorylation and internalization via Src kinase

A. VEGF activation induces VEGFR2 dimerization, enabling Src activation, which in turn phosphorylates VE-cadherin on Tyr-685 [71, 73]. Csk may associate with VE-cadherin phospho-tyr-685. This interaction was shown to inhibit proliferation in high density cell cultures [74]. Csk-VE-cadherin interaction may potentially inhibit VE-cadherin-associated Src by phosphorylating an inhibitory tyrosine in Src C-terminus domain.

B. Independently, VEGF was shown to induce an activation cascade (Src \rightarrow Vav2 \rightarrow Rac \rightarrow PAK kinase) leading to phosphorylation of VE-cadherin cytoplasmic domain on serine 665. Phospho-S665 VE-cadherin becomes a docking site for β -arrestin-2, the binding of which triggers VE-cadherin endocytosis and degradation, and thus, junction opening [76]. More work is necessary to examine the interplay between both pathways.

Fig. 6: Participation of VE-cadherin to VEGF signaling

It is clearly established that VE-cadherin has a pivotal role in contact inhibition of cell proliferation. VE-cadherin, through its interaction with Dep-1 or other tyrosine phosphatases inhibits VEGF proliferation signal via MAPK activation in three ways: (i) by reducing VEGFR2 phosphorylation, (ii) by inhibiting VEGFR2-associated Shc phosphorylation and (iii) by decreasing VEGFR2 internalization and signaling from endosomal compartments. VE-cadherin complex interaction with activated VEGFR2 also enhances VEGF survival signaling by PI3kinase/Akt pathway.

Fig. 7: Rap1 activation pathways and stabilization of the endothelial barrier

Increased levels of intracellular cAMP by G-protein-coupled receptors enable the activation of the exchange factor Epac, which is a specific activator of Rap1, a Ras family GTPase.

Rap1 activation promotes AJ extension by increasing the avidity of cortical actin to the VE-cadherin complex. Rap1 may also be activated by the adaptator protein MAGI-I together with the exchange factor PDZ-GEF1, recruited at the VE-cadherin complex. These data are derived from [128-130].

Fig. 8: Cytokines activating signaling pathways leading to occludin functional alterations (phosphorylation or proteolysis)

VEGF, MCP-1, lysophosphatidic acid (LPA) and histamine (Hist), by interaction with their cognate receptors, VEGFR2, CCR2, LPAR and HistR, respectively, induce increased occludin phosphorylation on serine/threonine residues, in correlation with increased permeability. Signaling pathways involve PKC activity, PKC α and/or RhoA, as indicated [163, 164, 167, 168]. VEGF also increases permeability by inducing occludin proteolysis through activation of the urokinase (uPA)/uPAR system [153, 160]. Conversely, angiotensin-2 (AT2) binding to type 1 angiotensin receptor (ATR) decreases permeability by inducing threonine phosphorylation and tyrosine dephosphorylation of occludin [169].

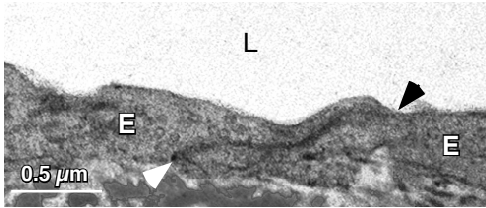


Fig. 1

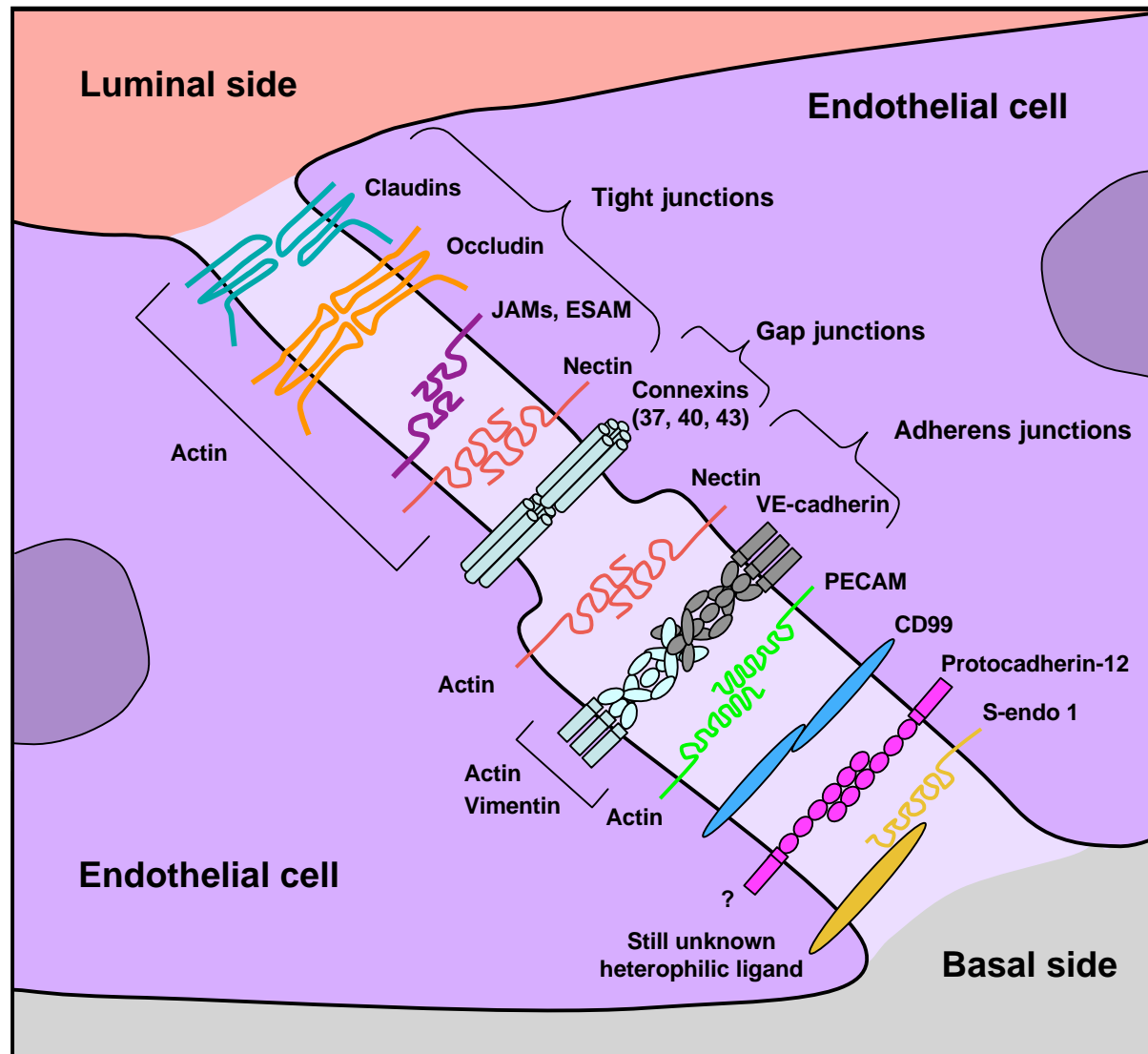


Fig. 2

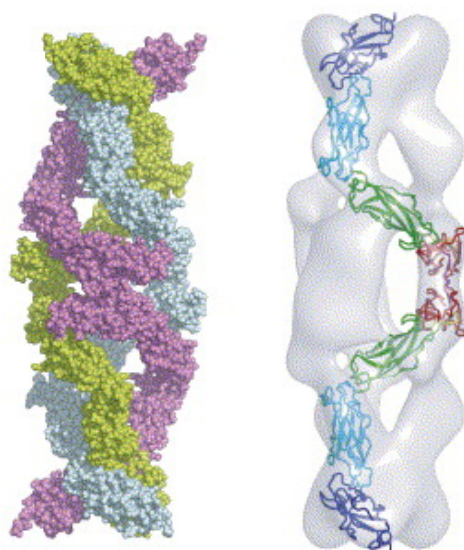


Fig. 3

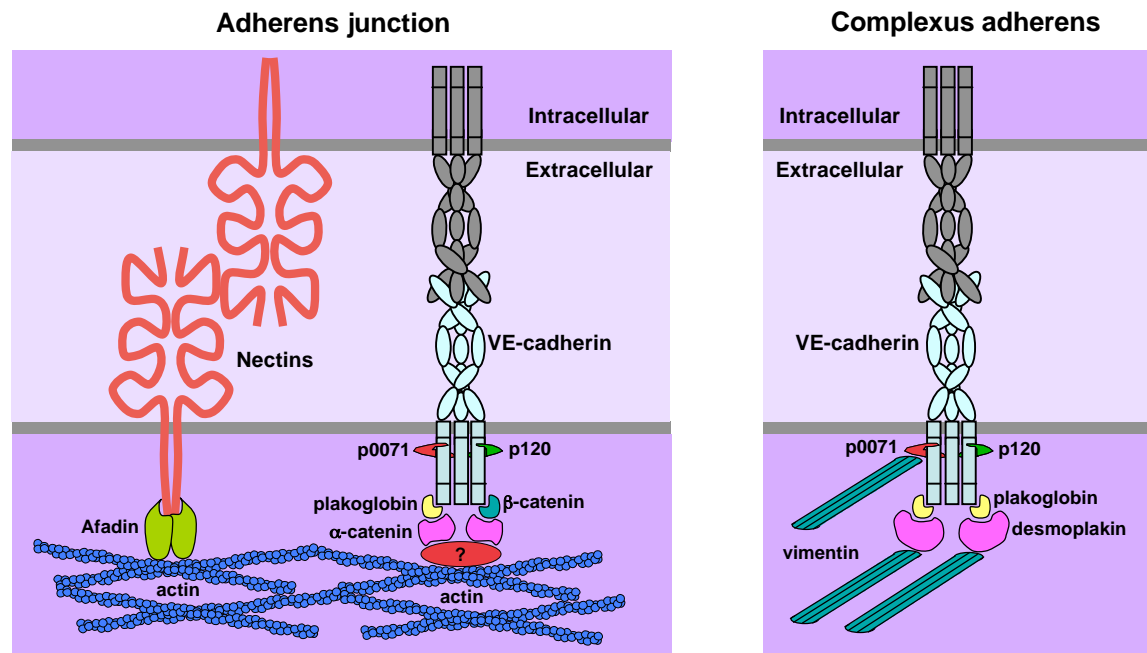
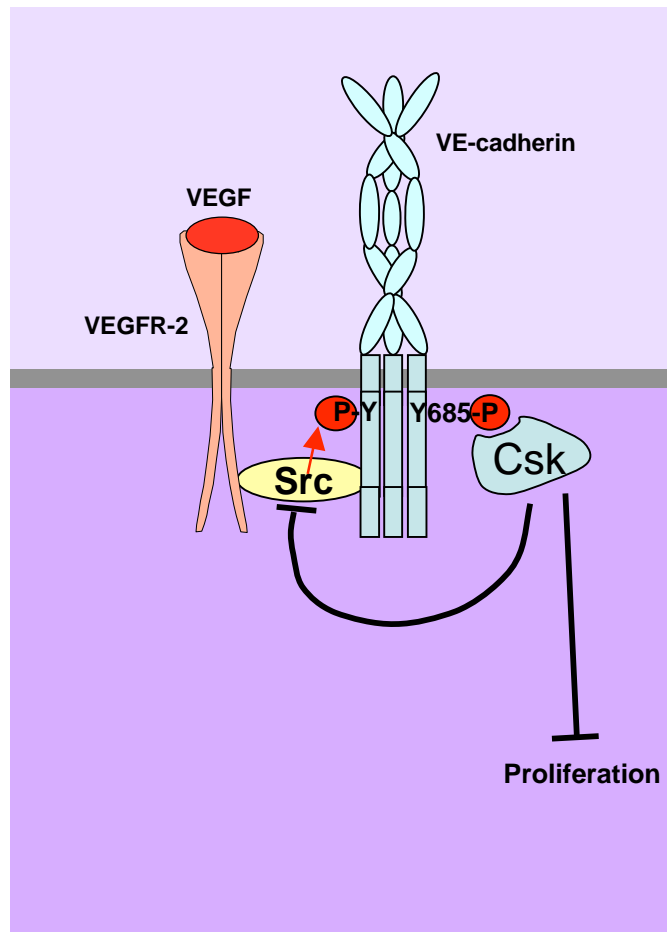


Fig. 4

A



B

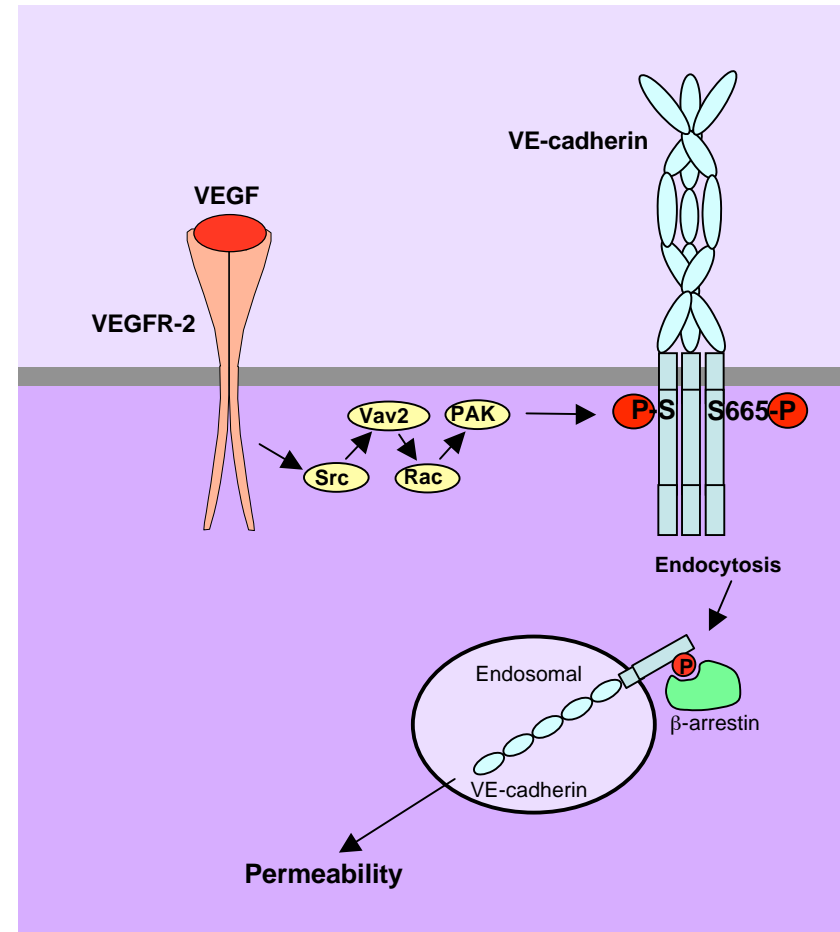


Fig. 5

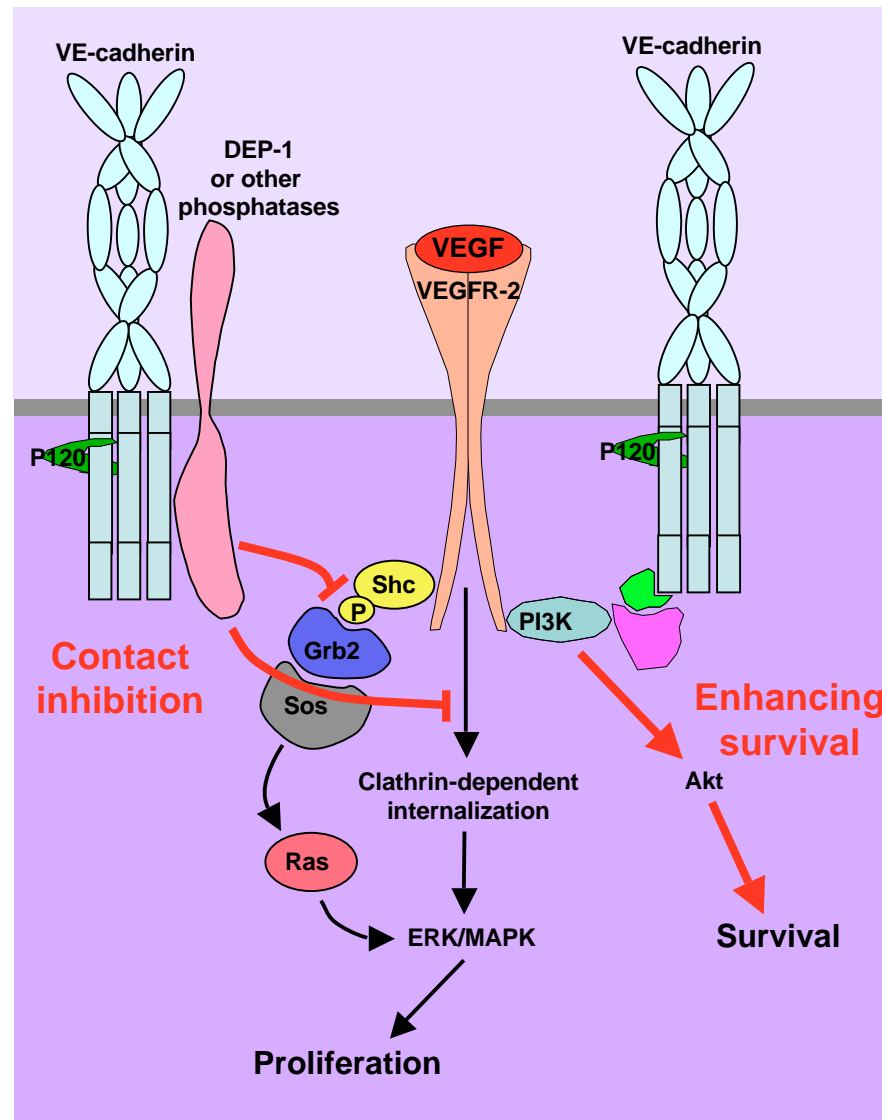


Fig. 6

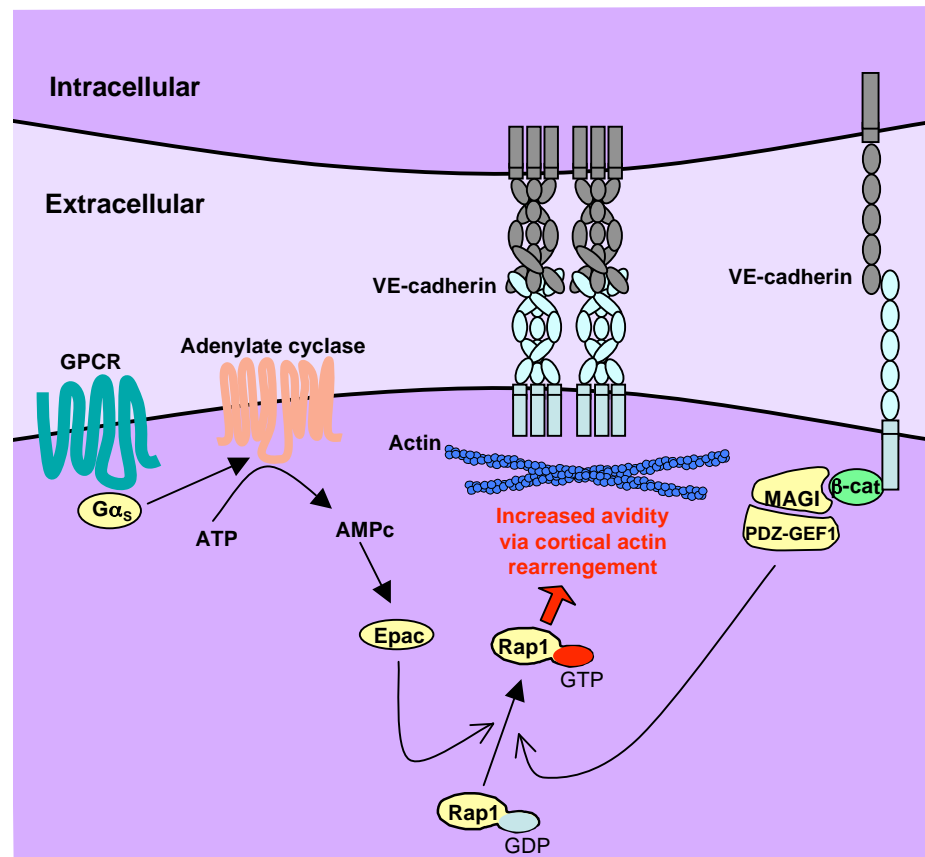


Fig. 7

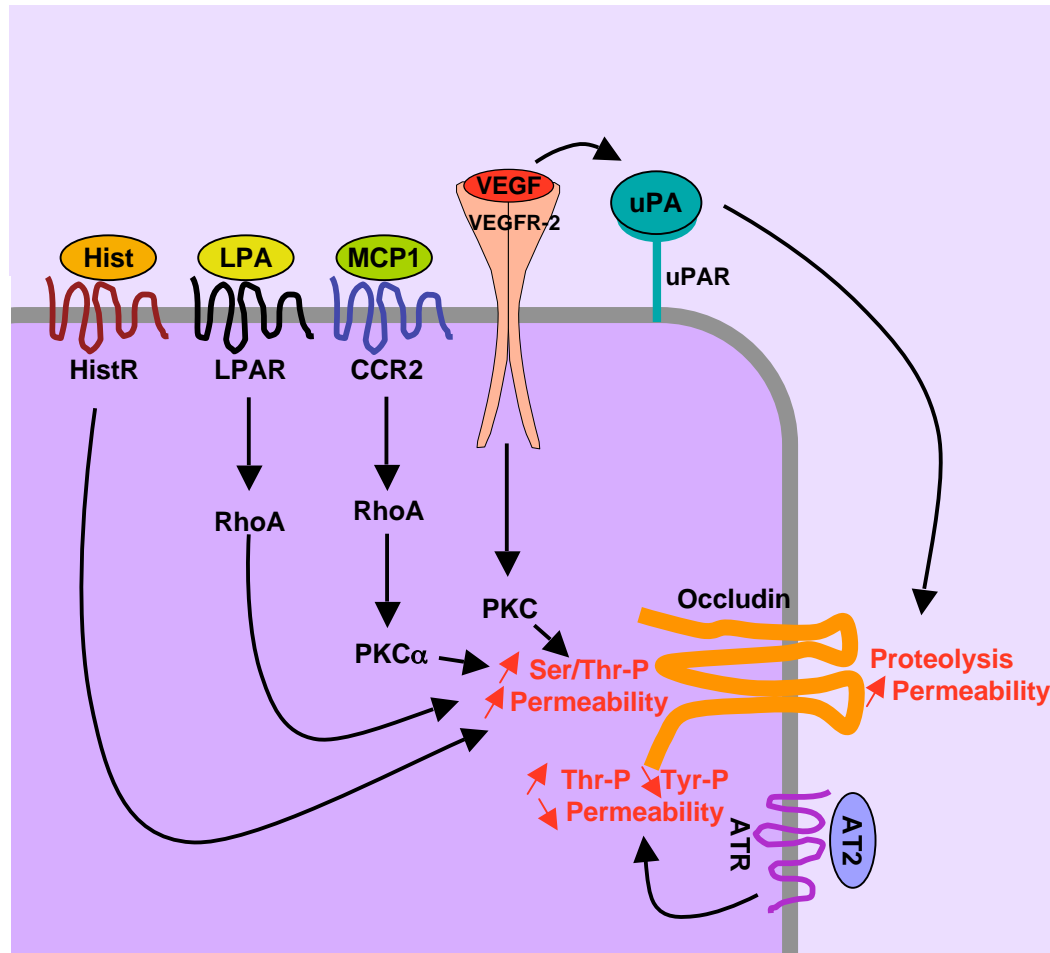


Fig. 8